

STUDIES ON THE CHEMISTRY OF AMIDES

and

MISCELLANEOUS MINOR INVESTIGATIONS

by

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Pt. 1.

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PREFACE

Rule 2. of the Degree of Doctor of Science requires the submission of published scientific work with notes on its sources, assistance given by other workers and the previous use of such publications towards the obtaining of other degrees. Copies of published work are attached to this Thesis. The required information is contained in the following list:

a) Publications which have been used to obtain a Doctor's Degree.

- 1) Absorptions. Spektrographische und chemische Untersuchungen über chemische Kampfstoffe. 95 pp. (Pápai, Budapest, 1937).
- 2) Hadigázok oldatainak abszorpciós spektrumai Magyar Chem. Folyóirat, 42 (4-9) pp. 1-7 (1936).
- 3) H. MOHLER and J. POLYA: Lösungsspektren chemischer Kampfstoffe. - Helv. Chim. Acta, 19, pp. 283-287 (1936).
- 4) H. MOHLER and J.B. POLYA: Chemische Kampfstoffe II. Lichtabsorption in Beziehung zur chemischen Konstitution. - Helv. Chim. Acta, 19, pp. 1222-1239 (1936).
- 5) H. MOHLER and J. POLYA: Chemische Kampfstoffe III. Spektroskopischer Nachweis. - Helv. Chim. Acta, 19, pp. 1239 - 1242 (1936).

1) has been submitted as a Thesis for the D. Sc. Tech of the ETH, Zurich; 2) contains suggestions concerning the correlation of colour and toxicity of war gases which are discussed more fully in 1); 3) is a preliminary publication; 4) and 5) contain the major theoretical

and applied results of 1).

Work reported in these publications has been initiated by Dr. H. Mohler. All experimental work, calculations, bibliography and toxicological portions are the author's work. The theoretical portions have been written in close collaboration with Dr. H. Mohler. The latter supervised the work.

3,4,5) have been drafted by Dr. H. Mohler and the author in close collaboration and appear with small changes in 1). The rest of 1) and the whole of 2) have been prepared by the author, the latter with the assistance of Herr J. Sorge.

b) Publications relating to work from which the author has withdrawn for the time being.

- 6) H. MOHLER and J. POLYA: "Über Kirschwasser V. Absorptionsspektrophotometrie des Wachses und der Wachssäure. - Zschr. Unters. Lebensmittel, 73, pp. 171 - 176 (1937).
- 7) H. MOHLER and J. POLYA: Absorptionsspektren einiger aromatischer Ester. - Helv. Chim. Acta, 20, pp. 96 - 100 (1937).
- 8) E. HAWORTH, I.M. HEILBRON, W.R. JONES, A.L. MORRISON and J.B. POLYA: The Oxidation of Vitamin-A by the Oppenauer Reagent. II. - J. Chem. Soc., 1939, pp. 128 - 132.
- 9) A.H. COOK, D.G. JONES and J.B. POLYA: cis-A₂o-compounds. III. Absorption Spectra. - J. Chem. Soc., 1939, pp. 1315 - 1320.

- 10) New Reactions of 2,2'-Dichlorodiethyl Sulfide. -
Ind. Eng. Chem., Anal. Ed., 15, 360 (1943).
- 11) Improved micro-method for the detection of nitrogen
in organic compounds. - Rev. Fac. Cienc. Univ.
Coimbra, 15 (1), pp. 5-8 (1946).
- 12) J.B. POLYA and O.G. INGLES: Removal of Bromine
and Iodine from Sugar Solutions. - Austr. Chem.
Inst. J., 14, 519 (1947).
- 13) E.M. TRAUTNER and J.B. POLYA: Notes on a Variation
of the Schotten-Baumann Reaction. - Aust. Chem.
Inst. J., 15, pp. 52-54 (1948).

In publications 6), 7), 8) and 9) the author's work was limited to spectrographic work, including both experimental work and discussion of the theoretical aspects of the results with the other authors. In 9) the spectrographical work was shared with D.G. Jones although the author was concerned with most of the reported spectrographic measurements. In all these cases the papers have been drafted by other authors. - The author suggested the idea reported in the short note 12); this was verified by O.G. Ingles and later by the author. - E.M. Trautner suggested the method described in 13) and he carried out the first experiment with cinnamic acid. The author checked this, improved the method and applied it to the other acids reported in the paper. The author collected the references and drafted the paper.

c) Publications on current research projects.

- 14) J.B. POLYA and P.L. TARDREW: Amides I. Preparation of Diacetimide. - J. Chem. Soc., 1948, pp. 1081-1083.
- 15) J.B. POLYA and T.M. SPOTSWOOD: Amides II. Acylation of Amides to Diacylimines. - Rec. trav. chim. 67, pp. 927 - 941 (1948).
- 16) J.B. POLYA and T.M. SPOTSWOOD: Amides III. Notes on the Separation and Assay of Some Diacylimines. - Austr. Chem. Inst. J., 15, pp. 445 - 446 (1948).
- 17) A.L.GOOD and J.B. POLYA: Extraction of Lignin by Acetonitrile. - Austr. Chem. Inst. J., 16, pp. 180 - 184 (1949).
- 18) J.B. POLYA and T.M. SPOTSWOOD: Acetic Propionic Anhydride. - J. Am. Chem. Soc., 71, 2398 (1949).
- 19a) J.B. POLYA and D.L. INGLES: Organo-cobalt Compounds. - Nature, 164, 447 (1949).
- 19b) D.L. INGLES and J.B. POLYA: Preparation of Organo-cobalt Compounds. - J. Chem. Soc., 1949, pp. 2280 - 2282.
- 20) J.B. POLYA and P.L. TARDREW: Amides IV. The Use of Diacetimide in the Perkin Reaction. - Rec. trav. chim., 68, pp. 566 - 572 (1949).
- 21) J.B. POLYA and T.M. SPOTSWOOD: Amides V. Ultraviolet Absorption Spectra of Diacylimines. - Rec. trav. chim., 68, 573 - 578 (1949).

All these publications have been initiated by the author who has also organized and supervised the reported

investigations, compiled the references and drafted the papers. The author is solely responsible for the theoretical portions of these papers. More than half of the analytical work in 17) has been carried out by the author. Analytical work relating to the other publications has been carried out by the co-authors or others whose assistance has been acknowledged in the papers.

All experimental work relating to new compounds or new procedures has been carried out by the author either in the first instance or in checking experiments. The rest of the work has been carried out in the author's laboratory under his direct instructions. - 19a) is fully covered by 19b). Thus the inclusion of a reprint of 19a) was not thought to be necessary.

d) Papers in course of publication.

- 22) P.DUNN and J.B. POLYA: Amides VI. Studies on Acetamide and Diacetimide. - Rec. trav. chim., 1950.
- 23) J.B.POLYA and P. DUNN: The Use of Acetamide in the Melostegmin Reaction. - Cancer Research, 1950.
- 24) J.B.POLYA and K.N.VALLANCE: Notes on the Chemistry of Alkali Lignin. - Roy. Austr. Chem. Inst. J., 1950.

These papers have been set up in print and are expected to appear late during 1950. The work in these papers has been originated by the author. All analytical determinations and physical measurements have been carried out by the co-authors except in 23). Most of the synthetic experiments, identification tests and some of the

meiostagmin tests (in 23) are the author's work who has also collected the references and drafted the papers. The figures of 22) and 23) have been drawn by P.Dunn. Other papers in course of publication are also submitted. Acknowledgements in respect of such papers will be made in the appropriate sections.

e) Popular articles on scientific subjects.

- i) Economics of Vitamins in Australia. - Austr. Food Manufacture, 11 (8), pp. 3 - 4 (1942).
- ii) Food and Cancer. - *ibid.*, 12, (7), pp. 3 - 4 (1943)
- iii) Vitamin C. - *ibid.*, 12 (8), pp. 3 - 7 (1943).
- iv) Notes on the etymology of some pseudo-Arabic scientific terms and on some Arab cosmologists of the pre-Copernican period. - Letters to the Editor, Austr. J. of Pharmacy, February and April, 1945.
- v) Life. - Focus, June 1947, pp. 7 - 9.

Reprints or photocopies of these articles are not available but they are not relevant for the purpose of this Thesis.

f) Other publications.

- vi) Chemical Education in Tasmania. - J. Chem. Ed., 26, pp. 82 - 85 (1949).
- vii) Guide to the Study and Profession of Chemistry, Hobart, 1949, II and 22 pp.

These publications are submitted partly because they have some bearing on chemical matters and partly to permit external examiners to assess the advantages and

disadvantages of carrying out scientific work, like the one submitted in the Thesis, in Tasmania. These publications have been compiled from official publications after consultations with teachers and chemists in Tasmania. "Chemistry as a Profession in Australia", a publication by the Australian Chemical Institute, supplied some material for vii). This pamphlet has been published by the Tasmanian Branch of the Royal Australian Chemical Institute. It has been distributed amongst teachers and pupils of Tasmanian secondary schools and interested members of the Institute in other States.

g) Unpublished work.

Work under this heading will be reported in the following parts:-

A. A Thesis entitled STUDIES ON THE CHEMISTRY OF AMIDES which deals with diacylimines, a new modification of the Perkin reaction, 1,2,4-triazoles and some biochemical investigations.

B. Miscellaneous minor investigations.

In the Thesis, and to a lesser extent in B, portions of previously published work will be reconsidered in the light of evidence gathered after the publications had been made. As regards the author's share in this work, the notes under 2c) apply, particularly in portions submitted in the form of manuscripts of papers which are in course of publication. In general, all theoretical considerations and bibliographic reviews

are the author's own work unless stated otherwise. Experimental work reported without acknowledgment represents work done by the author in the first instance or alone. Experimental work shared with a co-author will be indicated thus: with N.N.. Experimental work carried out by the author's students independently from the former will be indicated thus: by N.N.. Further experimental details may be obtained from Theses completed under the author's direction. A list of these Theses follows with initials which will be used for purposes of reference:

A.L. GOOD (A.L.G.) : An Investigation of the Extraction of Lignin from Tasmanian Timbers with Nitrogenous Reagents. --- Honours Thesis, 1947.

T.M. SPOTSWOOD (T.M.S.) : A Study of the Diacylimines. Honours Thesis, 1948.

A.D. DARGAVILLE (A.D.D.) : Investigations on the Oxidation of 1,2-Aminonaphthol. --- Honours Thesis, 1949.

P. DUNN (P.D.) : An Investigation of Some Aspects of the Biochemistry of Cancer and Other Related Problems. --- Honours Thesis, 1949.

N.K. VALLANCE (N.K.V.) : Lignin - A Study of Some of Its Reactions and Properties. --- Honours Thesis, 1949.

P.L. TARDREW (P.L.T.) : The Synthesis and Reactions of Diacylimines. --- Master's Thesis, 1949.

T.M. SPOTSWOOD : Studies in the Preparation and

Chemistry of Some Ethene Derivatives. --- Master's Thesis, 1949.

D.L.INGLES (D.L.I.) : An Investigation of Organocobalt compounds. --- Ph.D. Thesis, 1949.

Details of experimental work reported in publications and Theses will be given only when such repetition appears to be necessary to maintain the unity of treatment. At the time of submitting this Thesis work is in progress on a number of problems to which reference will be made in the following pages. Incomplete experimental work will be reported qualitatively only. It should be understood that in such cases full experimental data will be published as soon as possible.

References to work listed in the Preface are shown under different numbers, in order of occurrence, in sections with separate bibliographic lists. This somewhat inconvenient arrangement is due to the desirability of maintaining the identities of papers in course of publication.

In the major section of this Thesis the author has attempted a compromise between presenting the material in the form of a review of past and present work and arranging it to show the gradual development of the author's own work. The author wishes to apologize to readers who find this arrangement inconvenient and expresses the hope of having this section published in the form of a proper review.

The following grants are gratefully acknowledged:-

- i) Mr. E.J.Hallstrom, F.R.Z.S. : Grant for the investigation of some biochemical aspects of cancer and related problems.
- ii) Tasmanian Forestry Commission : Grants to investigate problems related to the chemistry of lignin.
- iii) Electrolytic Zinc Co. of A/sia Pty. Ltd. : Grant for investigations on cobalt.
- iv) Commonwealth Research Grants for investigations reported in Sections A and B.

Finally, the author wishes to acknowledge the help of Prof. E.E. Kurth, Mr. G.C. Israel and other members of the Chemistry Department who have discussed and criticized the author's work and eased the burden of administrative work involved in his investigations.

1st December, 1950.

J.B. Polya

A. STUDIES ON THE CHEMISTRY OF AMIDES .

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| J.B.Polya , T.M.Spotswood : The use of diacylimines in the Perkin reaction . | |
| J.B.Polya , T.M.Spotswood : Benzylidene and purylidene bisamides. | |
| J.B.Polya , P.Dunn : The use of acetamide in the melo-tagmin reaction. | |
| W.D.Jackson , J.B.Polya : Cytological effects of 1,2,4-triazoles I . | |

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A. STUDIES ON THE CHEMISTRY OF AMIDES

I. Introduction.

The larger part of this section is devoted to a preliminary survey of simple diacylimines. Karrer's Lehrbuch der Organischen Chemi~~k~~ provided the first incentive towards the investigation of this class of compounds with the words : "Es sind auch sekundäre und tertiäre Säureamide bekannt, die aber weniger Interesse besitzen". Relatively few compounds of this class have been described and even fewer were studied systematically from a chemical point of view, particularly during the last 30 years.

The well-known technical difficulties of the Gabriel synthesis of primary amines have led the author to consider variations of the method involving secondary amides (diacylimines) other than phthalimide. The first investigations arising from these considerations are reported in this section but it will be seen that the original problem leading to these investigations has received little attention so far.

Experimental work in this field has been initiated in 1946 when the author joined the staff of the University of Tasmania. At that time shortage of chemicals, equipment and assistants and the lack of analytical services called for research projects which could be pursued with a reasonable hope of success in spite of these handicaps. The study of diacylimines appeared to be a suitable subject from this point of view partly because of lack of detailed information on the most elementary features of the chemistry of this class of compounds, partly because the work was less dependent on analytical work than more spectacular studies on the chemistry of naturally occurring compounds and other

complex organic substances.

Preliminary investigations on diacylimines were carried out by the author alone in 1946. These investigations drew attention to the advantages of the acid catalyzed acylation of amides, the possibilities of the modified Perkin reaction, some aspects of the Brunner synthesis and the mild denaturing effect of diacylimines on proteins.

The first of these points was investigated with P.L. Tardrew in 1947, restricting the work to diacetimide alone (1). The preparation of other diacylimines was investigated with T.M. Spotswood in 1948 (2) with particular reference to the possible reaction mechanisms. An extension of the work to the preparation of N-substituted diacylimines was undertaken with P. Dunn in 1950. The main results of this work (which is being prepared for publication) will appear in this survey while other features of it, with which the author was not associated to any great extent, will be incorporated in a M.Sc. Thesis which is being drafted by P. Dunn.

The modified Perkin reaction between benzaldehyde and diacetimide has been studied with P.L. Tardrew (3) during 1948 and 1949. The work was continued with T.M. Spotswood during 1949 and 1950; two papers in course of publication and additional results appear in this survey.

The Brunner synthesis was investigated with P.L. Tardrew during 1948-1950 and later with M. Atkinson and Dr. A. Komzak. The chemical portion of the work cannot be considered complete. None of our workers could give undivided attention to this interesting problem so far but it is hoped that the first part of the work will be completed early in 1951. In the meantime some data

have been obtained with W. Jackson on the c-mitotic and anti-auxin effects of a few 1,2,4-triazoles.

On noting the mild denaturing effect of diacetimide, attempts were made to investigate its usefulness in melostagmin reactions (4). This work was carried out with P. Dunn during 1949 and with A. Parkes, occasionally assisted by P. Dunn, during 1950. Although diacetimide proved to be unsatisfactory, promising results were obtained with acetamide. This work necessitated the investigation of the structure and stability of acetamide and diacetimide. Work on this subject carried out with P. Dunn during 1949 is in press but later portions of the work are far from being complete.

Notes on the analysis and absorption spectra of diacylimines have been published with T.M. Spotswood (5, 6). Investigations with P.L. Tardrew on the modified Lipmann-Tuttle assay (7) adapted for use in the determination of diacylimines is in course of publication.

Synthetic experiments leading to the preparation of acetyl phosphamide have been carried out with G. Bratt during 1950. Publication of the results is being deferred pending checking experiments and biochemical investigations.

The oxidation, reduction and halogenation of some simpler diacylimines has been studied by the author. Notes on these reactions are intended to complete this survey rather than to present final and exhaustive data on these subjects. The following topics related to these investigations are receiving special attention at present:

Wohl-Ziegler reactions with N-bromodiacetimide

The "Meerwein reaction" with diacylimines.

A few preliminary data will be presented on the preparation and reactions of the N-magnesium bromide derivative of diacetimide.

II. Diacylimines.

1) Nomenclature and Classification.

Compounds of this class are defined here by the general formula $R-NXY$ where X and Y are acyl-groups and R represents hydrogen, alkyl, aryl or other groups. With R standing for an acyl-group we have triacylimines, a class of compounds with very few representatives, which will not be considered at present.

Before proceeding to a classification of diacylimines, the term itself deserves some attention. Since all compounds in this class may be regarded as acylated amides, the term diacylimide, cf. phthalimide, would appear more logical. Most of the work reported in this Thesis seems to stress this aspect of these compounds but projected work which has received little experimental attention so far will be more concerned with the amine portion and may justify the adopted term. Nomenclature facilitating the search for literature would have been desirable but the existing methods of classification defeated this aim. Thus Beilstein classifies diacylimines of the type $R.CO.NH.CO.R'$ under acids and those of the type $R.CO.NR''.CO.R'$ under amines ($R'' = \text{alkyl or aryl}$) or acids ($R'' = \text{acyl}$).

Although no particular sub-group of the class of diacylimines has a great many known representatives the whole class presents a scope which is beyond the power of experimental survey at a small institute. Purely inorganic diacylimines, e.g. $H_2O_3P-NH-PO_3H_2$ are unknown or their existence or structure are in doubt. These

will not be considered at all. Mixed organic-inorganic diacylimines are of great theoretical, and possibly practical, interest but only a few types of this kind will be considered in this work. N-haloamides may be considered as diacylimines if the halogen is regarded as the acyl rest of the corresponding hypohalous acid. Acetyl phosphamide, $\text{CH}_3\text{CO-NH-PO}_3\text{H}_2$ is the nitrogen analogue of acetyl phosphate. The biochemical importance of the latter has been recognized recently (8, 9) and its nitrogen analogue should be important also. Acylated sulphanilamides may be considered to belong to the same sub-group but will not be considered.

Organic diacylimines of the type $\text{R-CO-NR}^{\text{H}}\text{-CO-R}^{\text{K}}$ received most attention in this study. Here again it was necessary to restrict the scope of investigations by defining R^{H} and R^{K} as organic radicals linked through a carbon atom to the neighbouring (formal) CO group. This eliminates from our consideration acylated ureas and related compounds which, however, form one of the best investigated sub-groups of the class of diacylimines owing to their narcotic or sedative properties. If R and R^{H} are parts of the same organic radical, we have cyclic imides like phthalimide or succinimide. Compounds of this kind have received much attention in the past and continue to be of interest to many chemists. Since it is desirable to keep the review sections to a minimum, such cyclic imides will not be considered in detail. The chemistry of diacylimines derived from dicarboxylic or otherwise substituted acids (e.g. hydroxy- and amino- acids) present an almost virgin field to the investigator. It is proposed to examine such compounds at a later stage although reference will be

made to some halogenated diacylimines. At present most of our attention is centred on the simplest diacylimines with R and R' as alkyl or aryl groups. As indicated before, diacylimines with R'' other than hydrogen have received little attention so far; some preliminary work on such compounds will be presented in this Thesis.

As in the case of other classes of compounds, diacylimines will be termed symmetrical if R and R' are identical. If otherwise, they will be termed unsymmetrical or mixed diacylimines. Brief references to other compounds more or less related to diacylimines will be found in later sections.

2) Occurrence.

Diacylimines are comparatively stable and are readily formed. It is surprising therefore that no simple diacylimines have been isolated from natural products. Such an argument may be misleading, of course. Diacylimines are less soluble in water than the corresponding amides unless solubilized by additional functional groups. This almost excludes the probability of finding diacylimines in biological materials with the exception of a few simple diacylimines of low molecular weight, since solubilizing functional groups are likely to interact with the -CO-NH-CO- group. It is possible, however, that diacylimines play some role as intermediates in biochemical processes. Their formal similarity with anhydrides may facilitate the formation of energy-rich bonds without the biological disadvantages of the more reactive anhydrides.

Acyl phosphates are the only group of anhydrides which are commonly found in biological systems, although in very small

quantities only (8, 9). Advantage is taken of this fact in the assay of acetyl phosphate and similar compounds by Lipmann and Tuttle (7) who react the anhydridic compound with buffered hydroxylamine to obtain the fairly stable hydroxamic acid. The purple ferric chloride colour reaction of the latter is compared with a suitable standard. Under the described experimental conditions anhydrides only give a colour reaction of comparable intensity. Since the absence of ordinary anhydrides may be assumed, the test is considered significant. Details of the behaviour of diacylimines in this reaction will be given later but it may be mentioned now that diacylimines, as expected, react to hydroxamic acids and, if present in the biological system, would interfere with the assay of acyl phosphates. It is true that the optimum conditions for the assay of diacylimines are not quite identical with those of acetyl phosphate and related compounds but the new modification has been introduced merely to permit the assay of very insoluble diacylimines (e.g. acetyl benzamide) which are not likely to occur in biological systems. Soluble diacylimines like diacetimide can be assayed under the conditions set out by Lipmann and Tuttle. The author has carried out acetyl phosphate assays on defibrinated and centrifuged samples of normal and cancerous blood. The assays were conducted by the Lipmann-Tuttle method and were negative in every case. Repeating the assay with the modification of Polya and Tardrew (with a slight excess of alkali during the reaction with hydroxylamine) some sera from diabetic patients, but none from cancerous patients or normal persons, gave a faint but unmistakable colour reaction. There was not enough clinical material to present a final conclusion but it seems to be safe to say that the

samples concerned were free from the usual acyl phosphates and the modified test succeeded owing to the presence of a less reactive and/or less soluble compound of anhydridic character but different from the known acyl phosphates. This finding does not prove the presence of diacylimines in some diabetic sera but it supports the probability of finding such compounds in nature

The preceding arguments refer to true diacylimines only but it is justified to regard as diacylimines some other substances. Thus uracil, thymine, uric acid, xanthine and isoxaloxazines may be formulated as diacylimines. Cytosine, methyl cytosine and guanine may be formulated as acyl amidines which are clearly related to diacylimines. By the same argument and by synthetic evidence 1,2,4-triazoles may be considered as close relatives of diacylimines. Compounds of this class have not been reported to occur in nature but their close similarity to simpler azoles of biochemical importance should encourage work on the possibilities of competition between 1,2,4-triazoles and related compounds on either side, that is pyrimidines etc. with diacylimines character and azoles (imidazole and thiazole derivatives).

3) Preparation.

The methods for the preparation of diacylimines, with the imide function not contained in a cyclic structure, may be classified as follows:-

- a) Elimination of the elements of ammonia from two amide molecules;
- b) acylation of ammonia, amines, amides and other functional groups containing nitrogen;
- c) addition of carboxylic acids to nitriles;

A discussion of preparative methods must take in consideration structural problems. It might appear therefore that a discussion of the structure of diacylimines should precede that of preparative methods. It was felt, however, that a final solution of the problem of the structure of diacylimines cannot be offered at this stage of our knowledge and that an introduction to structural problems through the evidence of synthetic experiments would constitute a method of approach which has been amply justified throughout the history of organic chemistry.

a) Elimination of ammonia from two amide molecules.

Many pure amides of not too high molecular weight can be distilled without decomposition under atmospheric or reduced pressure. Although the boiling points of amides are high (e.g. acetamide, b.p. 222°), there is no evidence for the formation of diacylimines by the reaction



and condensation in the absence of catalysts is excluded.

The position is not quite as simple in the case of amides which boil with decomposition. Thus the attempted distillation of cinnamamide resulted in the formation of water, styryl cyanide, unchanged cinnamamide and unidentified tarry products. The formation of small amounts of dicinnamamide, $(\text{PhCH:CHCO})_2\text{NH}$, would have been difficult to prove by isolating this compound in a pure state from small scale experiments but extracts of all fractions of pyrolysed cinnamamide with alcohol, ether, petroleum ether, chloroform and hot water were tested by the original and modified Lipmann-Tuttle reaction without positive results. Benzamide boils without decomposition at 290° if the distillation is conducted with care. Rapid

heating of small quantities or superheating the vapours of benzamide afford small, varying quantities of dibenzamide and tribenzamide both of which give the hydroxamic acid reaction.

Strecker (10) obtained negligibly low yields of diacetimide by heating acetamide in a stream of hydrogen chloride. Acetamidine and acetonitrile were amongst the byproducts. The poor yields might have been due to lack of control over the quantity of hydrogen chloride and the reaction temperature. This method gave similar unsatisfactory results in the hands of Otto and Tröger (11) and Schmidt (quoted by Schulze, 12) and is of no practical value. Polys and Tardrew (1) controlled the quantity of hydrogen chloride by the use of bisacetamide hydrochloride, $2\text{AcNH}_2 \cdot \text{HCl}$, and the temperature by carrying out the reaction in ethyl acetate (b.p. 77°) and technical isoamyl acetate (b.p. $120-140^\circ$). In the former solvent refluxing for 20 hours did not affect the acetamide, 99% of which was recovered. The mother liquors from this experiment did not give the hydroxamic acid reaction by the original Lipmann-Tuttle method. (The modified method was avoided in this case so as to minimize the reaction of ethyl acetate). Refluxing in the higher boiling solvent for 2 hours afforded diacetimide (21.4%), acetamide and bisacetamide hydrochloride (4.8%) and ammonium chloride (85.5%). The yields are calculated on the assumption that the reaction can be represented by equation 21). The recovery of ammonium chloride is not far from quantitative. Loss of hydrogen chloride at $100-120^\circ$ (where the reaction proper does not take place) may account for the shortage of ammonium chloride. At the same time the recoveries of diacetimide and acetamide are significantly lower than that of ammonium chloride and indicate the occurrence of unwanted

side-reactions. In fact acetonitrile (31.2 - 43.2%), could be isolated by fractionating the low boiling distillates and small amounts of acetamidine (0.9 - 1.3%, as picrate) were isolated from the high boiling residue. The recovery of the two latter compounds was not quantitative since attempts to obtain pure products reduced the yields. One may summarize the results of these experiments as follows:

- i) In the presence of hydrogen chloride, but not without it, and at temperatures above 120° acetamide affords diacetimide with elimination of ammonia.
- ii) Side-reactions, particularly the formation of acetonitrile, predominate and thus the reaction is not suitable for preparative purposes.

There is no advantage in using a higher concentration of hydrogen chloride, e.g. by substituting acetamide hydrochloride, $\text{AcNH}_2 \cdot \text{HCl}$, for bisacetamide hydrochloride, as the former loses hydrogen chloride readily to give the latter. This occurs even in chloroform solution at room temperature at a rate of loss of about 2% hydrogen chloride per hour. Experiments with bisacetamide hydrochloride do not indicate whether the hydrogen chloride acts as a catalyst or whether it is required in quantities equimolar with the ammonia liberated in reaction al). Further experiments have shown that very little hydrogen chloride is required ($1/100 - 1/20$ mol for 1 mol amide) and that an increase of the hydrogen chloride concentration reduces the yield of diacylimine through the formation of nitrile. This is particularly noticeable when acetyl chloride or thionyl chloride are used as sources of hydrogen chloride. In these cases, however, condensation of two amide molecules is not the

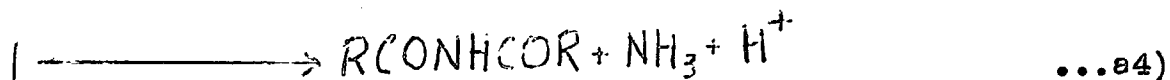
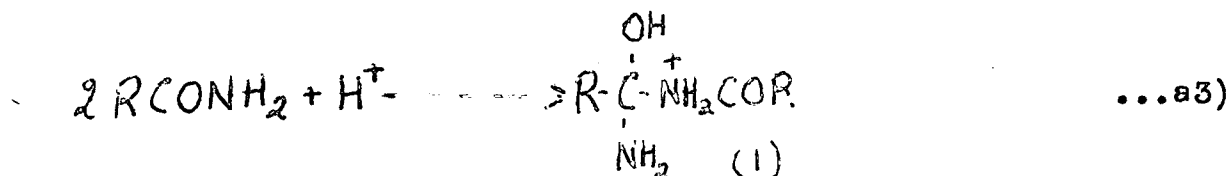
only reaction which takes place, and ~~the effects of~~ the effects of these and similar compounds will be discussed in later portions of this chapter.

Polya and Spotswood (2) have found that unsymmetrical diacylimines are the main product when two different amides (e.g. acetamide and chloroacetamide, benzamide and chloroacetamide) are heated together in the presence of less than 1/100 mol hydrogen chloride. When acetamide, chloroacetamide and a trace of chloroacetamide hydrochloride, $\text{ClCH}_2\text{CONH}_2 \cdot \text{HCl}$, are heated in xylene, which does not dissolve the latter two compounds but permits the decomposition of chloroacetamide hydrochloride into chloroacetamide and hydrogen chloride above $120-125^\circ$, the unsymmetrical diacylimine is formed in traces only and the main product is diacetimide. In any case the yields are poor in comparison with those afforded by the "acylation" of amides and the importance of this reaction is due to the fact that it participates to some extent in processes which were regarded as pure acylations so far.

Equation a1) does not describe the reaction correctly. If it occurred at all, good yields of diacylimines could be obtained without using hydrogen chloride, merely by conducting the reaction at a high temperature and allowing the ammonia to escape from the reaction mixture. However no formation of diacylimines could be observed when acetamide, propionamide and benzamide were heated to $180-200^\circ$ for 18 hours. Since yields of diacylimines of the order of 20% may be obtained with less than 1% hydrogen chloride, the reaction cannot be represented by the equation:



unless it is assumed that the ammonium chloride decomposes fast enough at the reaction temperature to make the limited amount of hydrogen chloride available again. This assumption is excluded by the fact that ammonium chloride is quite stable at the temperatures employed for the reaction (120-140°). Furthermore when the amount of hydrogen chloride is not sufficient to neutralize the ammonia formed during the reaction, evolution of ammonia may be observed. In view of these facts the reaction is regarded as analogous with the (formal) replacement of the amino groups of amides by hydroxyl, alkoxyl, hydrazine, hydroxylamino etc. groups, in this case by acylimino groups. Such reactions are reversible and formally similar to the reversible reactions of esterification and hydrolysis. The mechanism may be analogous with the Lowry mechanism of esterification (13, 14):

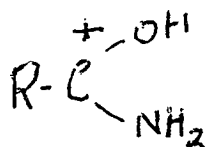


Equation a4) is in good agreement with the following:

- i) hydrogen chloride acts as a catalyst;
- ii) ammonia is evolved;
- iii) elimination of RCOOH instead of ammonia accounts for the formation of amidines;

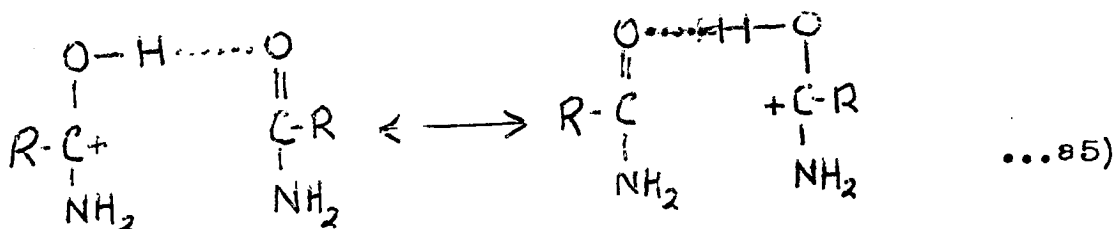
Equation a3) is unlikely in this simple form as it represents a termolecular reaction. It is plausible to assume the inter-

mediate formation of the carbonium ion



(II)

which then reacts with a further molecule of amide. This assumption presents no difficulties when there is only one amide in the reaction mixture. When the reaction is applied to the preparation of unsymmetrical diacylimines one would expect the more basic amide to compete successfully for a limited supply of protons to form II. The latter again would be expected to react with the more basic amide, that is its own kind. In this way one would predict the formation of symmetrical diacylimines which is contrary to experimental findings. This discrepancy may be explained by considering that one proton polarizes several molecules of amides in its neighbourhood. As II approaches an amide molecule of its own kind, the proton will be "kept in equilibrium" by resonance

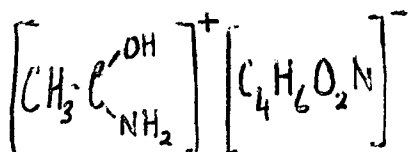


and I will not be formed with the same ease as when II approaches the less basic amide which cannot "tear away" the proton. This view is confirmed by the observation that the formation of unsymmetrical diacylimines by the reaction under discussion takes place with much better yields (44-48%) than the analogous

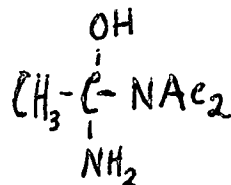
formation of symmetrical diacylimines.

The existence of compounds like $2\text{AcNH}_2 \cdot \text{HCl}$, $2\text{AcNH}_2 \cdot \text{NaBr}$, $2\text{AcNH}_2 \cdot \text{NaI}$ (15) and similar complexes of aromatic amides (16, 17) is readily explained by the postulate of I and related structures although the bonding of sodium cannot be allocated safely to the nitrogen on the available evidence. The analogy of such compounds with some addition compounds of esters (18-21), strengthens the validity of the fundamental assumption underlying these arguments.

The existence of $\text{Ac}_2\text{NH} \cdot \text{AcNH}_2$ (22, 23) can also be explained through a structure resembling I. Diacetimide is more acidic than acetamide and it may supply the proton necessary to form II. The subsequent step may lead either to a salt (III) or a covalent compound (IV):



(III)



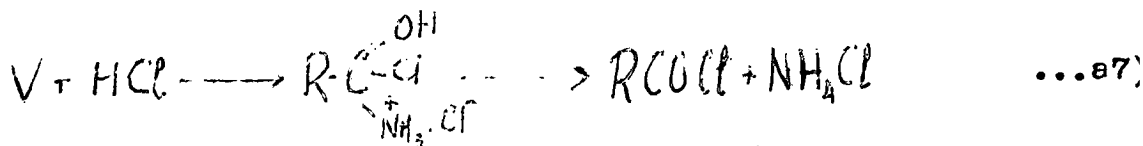
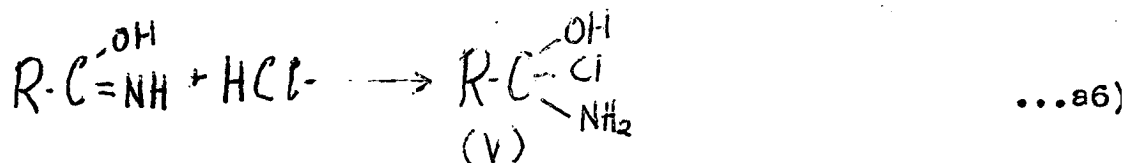
(IV)

When $\text{Ac}_2\text{NH} \cdot \text{AcNH}_2$ in ethereal solution is treated with dry hydrogen chloride - Strecker's method (10) - bisacetamide hydrochloride precipitates and diacetimide remains in ether. This reaction might suggest structure III if it is interpreted as the stronger acid (hydrogen chloride) driving out the weaker acid (diacetimide) from its salt. On the other hand if this interpretation were correct one would expect the formation of acetamide hydrochloride, which is contrary to experimental findings, and the considerable

solubility of a salt in ether would be difficult to understand. Thus IV appears more probable than III. The stability of the compound in ether and dissociation into acetamide and diacetimide in less polar solvents (petroleum ether, carbon tetrachloride) offers further proof against the ionic structure III but at the same time suggests that formulation with a comparatively strong dipole linkage might be preferable to IV.

The formation of bisacetamide hydrochloride from an ethereal solution of acetamide treated with dry hydrogen chloride is preceded by a time lag of 10-30 minutes. The reaction is complete soon after the appearance of the first cloudy precipitation and is quantitative. When chloroform is used as a solvent instead of ether there is little or no time lag. The precipitate in this case consists of acetamide hydrochloride which is not obtained in quantitative yield in this case since it is appreciably soluble in chloroform. The time lag in ether solution may be explained through the successful competition of ether (a stronger base than acetamide) for protons. Significant formation of I and then II does not take place until the ether - hydrogen chloride - oxonium salt system reaches or approaches equilibrium. The great excess of unchanged amide over II promotes the formation of I. The chloride of I precipitates and more I is formed to maintain the equilibrium of the amide - II - I system. In chloroform solution acetamide is more basic than the solvent. This leads to a rapid formation of II and inhibition of the formation of I through the comparative shortage of unchanged amide. Hence the precipitate is the chloride of II rather than that of I. However the formation of I is not completely inhibited since analysis of the

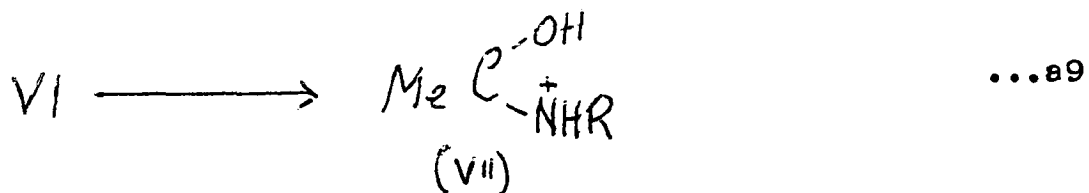
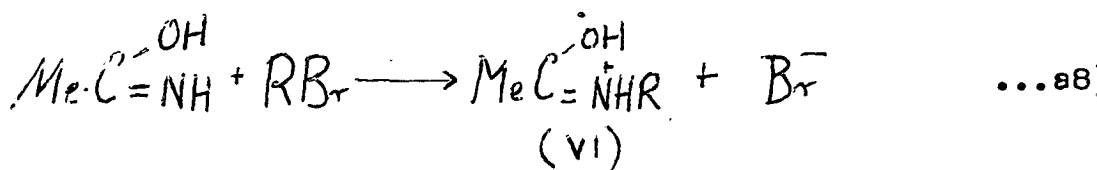
acetamide hydrochloride obtained by this method gives chlorine figures 2-3% below the calculated percentage. Propionamide, which is more basic than acetamide, affords the amide hydrochloride, $\text{EtCONH}_2 \cdot \text{HCl}$, from ether in agreement with these arguments. Butyramide precipitates incompletely as the amide hydrochloride, $\text{PrCONH}_2 \cdot \text{HCl}$, from ether while isobutyramide does not precipitate as hydrochloride from organic solvents. Evaporation of ether solutions of the latter amides after treatment with dry hydrogen chloride yields mixtures of varying composition which contain small amounts of ammonium chloride and acyl halides in addition to unchanged amides. These facts may be explained by assuming that the importance of the imidol form is greater in butyramide and isobutyramide than in acetamide and propionamide and that the following reactions take place with the imidols:

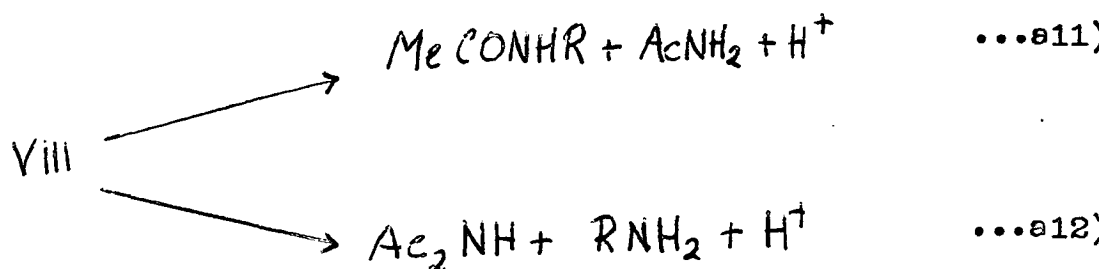
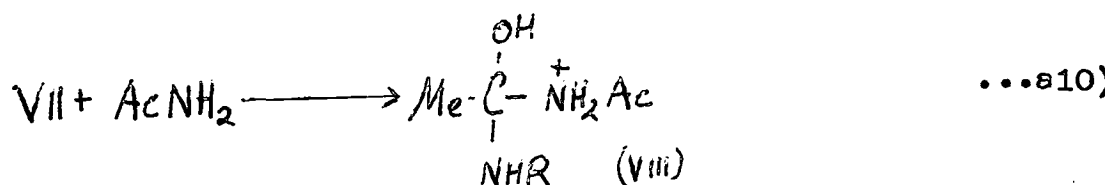


The same considerations apply to benzamide which may react in the amide and imidol form. Treatment of its ethereal solution with dry ~~gas~~ hydrogen chloride in a very fine stream with good stirring gives a slight precipitate of $\text{PhCONH}_2 \cdot \text{HCl}$ which dissolves when excess hydrogen chloride is passed into the solution (experiment by T.M.S.). If the solution is evaporated under reduced pressure

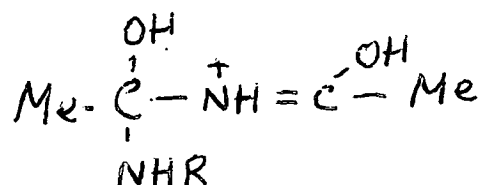
the residue treated with liquid ammonia and allowed to evaporate to dryness, benzamidine may be recovered in poor yields (2-8%). This indicates the appearance of the iminochloride as an intermediate the formation of which is more plausible through the imidol form. The transitory formation of $\text{PhCONH}_2 \cdot \text{HCl}$ may be explained equally well through the amide or the imidol form but the latter is more in agreement with the views of Titherley (24-26). The remark of Titherley (24) that acetamide fixes hydrogen chloride while benzamide does not is still correct for the purpose of his arguments although it must be interpreted somewhat elastically in view of our experiments. This matter will be considered later in another connection.

The formation of diacetimide from acetamide and alkyl bromides at $200-220^\circ$ observed by Nicholas and Ericson (27) probably occurs through a mechanism related to the one under consideration. This reaction has not been investigated experimentally by the author. The hypothetical equations a8) - a12) (from the imidol form) and a13) - a14) are offered merely to suggest that the proposed mechanism a3) - a4) may be applicable beyond the original scope of the author's investigations.

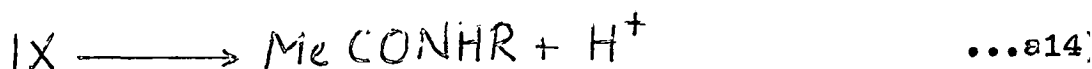
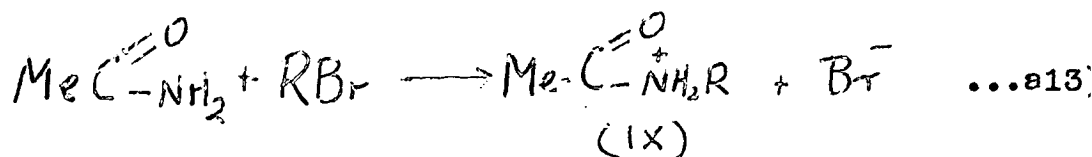




(Note that VIII could be formulated as



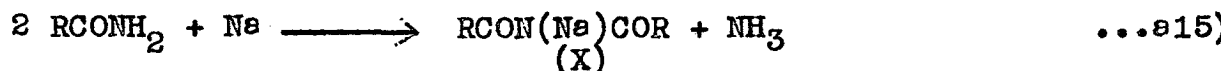
to give consistently the, unlikely, imidol structure to acetamide in the reaction.)



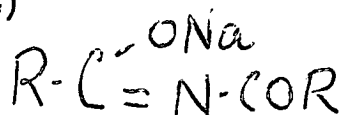
The protons liberated according to equations s11), s12) and s14) would turn the system into one to which equations s3) and s4) are applicable. Thus the hypothetical mechanisms could take place to a very slight extent only and yet permit the catalysed condensation of acetamide which has been shown to occur with as little as

1/200 - 1/100 mol hydrogen chloride.

Finally we shall consider the condensation of amides to diacylimines by heating with alkali metals in inert solvents. This method has been originated by Rakshit (28, 29, cf. 30) whose claims are represented by equations a15) - a16)



Parts has criticized Rakshit's work (31) and his criticism has been confirmed by the author's experiments (partly with P.D.). The formation of the sodium derivatives of diacylimines is accompanied by that of the sodium derivatives of the amides, $(\text{RCONH})\text{Na}$, which account for 80-95% of the product in the case of propionamide and the isomeric butyramides and not less than 60% in the case of acetamide. The mixture of sodium compounds cannot be separated. Dissolving in cold ethenol and precipitating with ether seems to be the most satisfactory method but the product is always contaminated by sodium ethoxide. The sodium compounds of diacetimide, acetyl propionamide and dipropionimide are much more readily hydrolysed by water to the corresponding acids (as ammonium and sodium salts) than the diacylimines themselves. This may be due to the high concentration of sodium hydroxide formed through the simultaneous hydrolysis of sodium ethoxide. An alternative or additional explanation may be that the sodium in Rakshit's products is attached to oxygen rather than nitrogen (32, cf. 33, 34)



which would enhance hydrolysis (35). Attempts to prepare N-methyldiacetimide and triacetimide from "sodium diacetimide" obtained by Rakshit's method have failed. It will be seen, however, that the well characterized and pure N-magnesium bromide derivative of diacetimide could not be used in analogous reactions either. The preparation of dihomoverstroimide or its sodium derivative by Rakshit's method (with T.M.S.) was unsuccessful.

A possible improvement of the method would consist of preparing sodium amides (36, 37) and reacting them with free amides in boiling toluene and xylene. A few orienting experiments of this nature have been carried out but using reactants in M/10 amounts did not afford diacylimines in amounts sufficient to permit isolation in a pure state. However the modified Lipmann-Tuttle test proved the formation of diacylimines in small quantities. Comparing the colour intensities qualitatively only, it is suggested that the modified Rakshit method is somewhat more successful when reacting the sodium derivatives of benzamide and the isomeric butyramides with aromatic amides. There is considerably less diacylimine formation when the sodium derivatives of acetamide and propionamide are used. Benzamide does not appear to react to form diacylimines with the sodium derivatives of the investigated aliphatic amides. Since the sodium derivatives of diacylimines (prepared by the direct reaction of sodium powder with diacylimines) give the Lipmann-Tuttle test, the conversion of the crude mixture of sodium compounds to diacylimines by alcoholic ethanol was omitted in order to avoid the possible hydrolysis of the traces of diacylimines formed in this reaction.

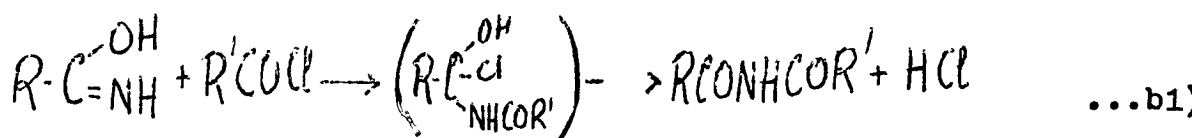
Decomposition of sodium derivatives of diacylimines with

ethanol is impracticable in Rakshit's original method since the crude product always contains some sodium protected by a layer of sodium amide and/or sodium diacylimine. As will be shown later, diacylimines are reduced by sodium and alcohol and the same has been known about amides for some time (38, 39). Reduction to amines, although on a much smaller scale, occurs in the modified Rakshit process. This may be due to the occlusion of traces of sodium in the sodium amides or else a Dumas-Stas reduction (40, 41) may occur to some extent. These qualitative observations deserve further study since they tend to confirm the hypothesis of the enhanced imidol character of benzamide and the higher aliphatic amides. Lack of time and adequate physical equipment has prevented the extension of the studies on the various implications of the original and modified Rakshit reaction but it is hoped to devote some time to these problems in the near future. It will be seen that our qualitative experiments on the modified Rakshit reaction gave results similar to those obtained by Titherley in his more fully studied work on the reaction between sodium derivatives of amides and esters (42). It would appear from that work that the presence of $-\text{CH}_2-\text{CO}-$ groupings either in the ester or the sodium amides inhibits the reaction. One possible implication of this will be considered later, after evolving further arguments leading in the same direction. Modifications of the Rakshit reaction are more conveniently classified as acylations which will be considered in the next part of this chapter.

b) Acylation of ammonia, amines, amides and other functional groups containing nitrogen.

The acylation of ammonia by acyl halides is commonly used to prepare amides (43-47). Hofmann (48) noted the formation of diacylimine⁶ with isobutyryl chloride. The formation of triacylimines was not noted by Hofmann in his series of experiments but Jaffé¹ (49) observed the formation of dibenzamide and tribenzamide from ammonium carbonate and benzoyl chloride. Although this method is not of great importance for the preparation of diacylimines, it is of historical interest. On the theoretical side two features of this reaction deserve mention. A direct reaction of two molecules of acyl halide with one molecule of ammonia (or ammonium carbonate) to give a diacylimine is as unlikely as most other reactions which appear termolecular in their simplest formulation. The assumption that amides are formed first and react subsequently with a second molecule of acyl halide to diacylimines (also with a third molecule of acyl halide to triacylimines in the case of some aromatic amides) is plausible but admits a number of more detailed explanations. One of these is that acyl halides acylate by a mechanism analogous to that of anhydrides. Such an analogy exists in the case of Friedel-Crafts acylations (50-59). The present work was concerned mainly with the mechanism of acylations by anhydrides. Should the assumption of an identical mechanism of acylation prove correct at some later time, the arguments evolved with reference to anhydrides will apply to acyl halides also. However it will be shown in this section that there is some reason to believe that this assumption does not apply entirely to acylations of amides by acyl halides.

A second possibility (49) is the dehydration of amides to nitriles with simultaneous hydrolysis of the acyl halides to acids and hydrogen chloride followed by a Gautier reaction (22). This will be considered in part c) of this chapter. A third possibility is the addition of acyl halides to the imidol form of amides with subsequent elimination of hydrogen chloride



This assumption is in agreement with ideas of Nef (60) on the general reactions of C=N functions. Nef did not consider the implications of his theories from the point of view of diacylimine syntheses but similar views have been expressed by other workers engaged in the study of diacylimines (25, 61, 62). In section a) arguments have been offered in favour of the increased importance of the imidol form in isobutyramide and benzamide. The increased yields of diacylimines from reactions between ammonia and acyl halides with likely intermediates of amides for which an increased imidol character is postulated lend some support to this theory. There are empirical facts in favour of all three mechanisms. These facts are not numerous enough to decide the matter but it is suggested, on heuristic grounds, that all the three mechanisms participate to some extent in the formation of diacylimines (and occasionally triacylimines) from ammonia and acyl halides.

The ammonolysis and aminolysis of esters under pressure (63-70) is a method of industrial importance for the preparation

of amides. In the absence of suitable pressure equipment, only a few small scale experiments of this kind have been carried out by the author. Attempts to prepare N-formyl amides by heating acetamide or benzamide with ethyl formate to 180-200° for 4 hours failed. A similar reaction between acetamide and ethyl acetate afforded mostly unchanged products which gave a faintly positive Lipmann-Tuttle test. In the preparation of amides by this method ammonium chloride was found to have a catalytic effect (66-67). Diacylimines are not formed in this reaction, at least not in significant quantities. This indicates that in the amide condensations leading to diacylimines, which have been discussed under a), the ammonium chloride formed during the reaction is not a catalyst.

Brunner and his collaborators obtained diacetimide by treating potassium cyanate or thiocyanate with acetic anhydride (32, 71-73; cf. 74), Brunner's experiments were repeated by the author who was able to confirm the yields of 56-74 parts of diacetimide from 100 parts of potassium cyanate and 34-41 parts of diacetimide from 100 parts of potassium thiocyanate (Brunner : 70-80 and 32 parts diacetimide respectively). An extension of this reaction to other anhydrides, particularly unsymmetrical anhydrides, would be desirable but shortage of time and the ready availability of diacylimines by cheaper methods moved the author to defer such experiments for later study. A similar method has been recommended for the preparation of N-aryl diacylimines by reacting aryl mustard oils or isocyanates with anhydrides (75-78). It will be shown in this section that a cheaper and more convenient method can be used in some cases. Hofmann's method of

preparing diacetimide by acylating N,N'-methyl acetyl urea with acetic anhydride (33) is closely related to the acylations of cyanates and thiocyanates but it is of too restricted scope to warrant detailed discussion.

On comparing yields, expense and convenience of technique, it appears that the best method for the preparation of diacylimines, free from N- substituents, is the acylation of amides by anhydrides or acyl halides. Reactants in these classes are readily available. The experimental technique is simple and good yields are obtained in many cases. The first attempt at a synthesis of this nature is due to Lindemann (79) who reacted acetamide and acetic anhydride with sodium acetate under pressure. This technique was simplified somewhat by Franchimont (80) but neither of these authors was able to obtain significant yields of diacetimide. Hentschel (81) investigated this reaction in detail and worked out a preparative method which was not improved until recently. Before considering the theory of this reaction, the technique of Hentschel's diacetimide preparation and that of our modification (1, 23) will be discussed in some detail since most of our diacylimine preparations are derived from this method.

Hentschel showed that sodium acetate has no catalytic effect on the acylation of acetamide by acetic anhydride and that the raised temperature of the reaction mixture (through the presence of sodium acetate) is deleterious since higher reaction temperatures decrease the yield of diacetimide by forcing the reaction



to the right. Hentschel also drew attention to the importance of controlling reaction times. He gave 30 minutes as the optimum

time for the initial refluxing of acetamide and acetic anhydride. This has been confirmed by the author and his assistants in a very large number of diacetimide preparations which seem to indicate that as little as 10 minutes' increase or decrease of the reaction time recommended by Hentschel depresses yields by 20-30%. The second step in Hentschel's procedure is the distillation of the lower boiling fractions of the reaction mixture (mainly acetic acid with a little acetonitrile and some acetic anhydride carried over by the vapours of the former two) under atmospheric pressure up to 125°. Hentschel claimed that this part of the process is essential to complete the reaction. In confirmation of this the author found that the yield decreases if distillation is carried out in vacuum at this stage. The yields of higher diacylimides do not appear to be affected by the substitution of vacuum distillation for distillation under atmospheric pressure. The time taken for the atmospheric distillation is expected to affect the yield of diacetimide. Hentschel did not consider this in detail. Variable results were obtained by the author and his assistants. From our present experience, it is suggested that 30 minutes for a 3-5M preparation is satisfactory. The third step in the Hentschel process is the removal of the excess acetic anhydride in vacuum followed by the collection of diacetimide (with varying quantities of $\text{Ac}_2\text{NH} \cdot \text{AcNH}_2$). This step presents no difficulties if care is taken to avoid both the clogging of the condenser by the solids and sublimation from the receiver.

Hentschel purified the crude diacetimide by Strecker's hydrogen chloride method (10). This method was followed by the author in earlier experiments. It was abandoned later owing to

a number of difficulties. Experiments with P. Dunn have shown that an ethereal solution of diacetimide may be converted quantitatively to bisacetamide hydrochloride by prolonged treatment with dry hydrogen chloride. Since the precipitation of acetamide present in the crude product is slow,,the hydrogen chloride has time to decompose some diacetimide before the precipitation of the original acetamide is complete and it is very difficult to judge how to compromise between requirements of yield and purity. In the author's experience the precipitation of the original acetamide is complete in about 30 minutes in 3-5M experiments. While the hydrogen chloride generator is disconnected and during filtration there is some secondary precipitation of bisacetamide hydrochloride presumably due to the gradual decomposition of diacetimide. After a rapid second filtration and immediate neutralization of the excess hydrogen chloride further decomposition of diacetimide is avoided and diacetimide of correct analysis is obtained. The neutralization, however, presents further problems. Dry potassium carbonate or barium carbonate react too slowly. Addition of a few drops of water hastens the neutralization of hydrogen chloride but a little hydrolysis takes place during this process. With some experience large amounts of diacetimide of good melting point and analysis were obtained in good yield by this method of purification but further physical measurements drew attention to the unsatisfactory nature of the product (4, 23). Diacetimide purified by Strecker's method hydrolyzes much faster in cold water than diacetimide purified by recrystallization from petroleum ether. This is not due to traces of retained hydrogen chloride or acetic acid since there is no significant change

between the pH values of fresh aqueous solutions of the two products. 5% solutions of diacetimide purified by Strecker's method have surface tensions varying around 50 dyne/cm (at 18°) as against the readily duplicated value of 67.8 dyne/cm for diacetimide solutions of the same strength and at the same temperature prepared from material purified by recrystallization from petroleum ether. When the unstable solution is brought to pH 9 by the addition of potassium carbonate a high melting substance of unknown composition precipitates in small quantities while solutions of stable diacetimide do not give this reaction. Purification by petroleum ether gives lower yields than those published before (1) but this method deserves preference when diacetimide is required for critical physical and biological measurements.

It has been noted by Polya and Tardrew that hydrogen chloride acetyl chloride or the hydrochlorides of acetamide considerably increase the yield of diacetimide from Hentschel's procedure (1). Our first experiments of this kind were undertaken on the assumption that such reagents may afford diacetimide from a catalyzed condensation reaction in addition to that produced by a straight acylation of acetamide by acetic anhydride. It will be shown later that this assumption was not quite correct. Before proceeding to a discussion of the mechanism of the Hentschel process the preparation of diacetimide by the present process used in the author's laboratory may be of interest. This process was established by the author. It has been checked with P. Dunn (23) and later in greater detail by Dr. A. Komzek whose modifications are quoted as follows:

Starting materials:

Acetamide, distilled at 131-133°/24-25 mm; m.p. 80-81°;

Acetic anhydride, freshly distilled, b.p. 138-140°;

Acetamide hydrochloride, prepared according to (1).

Each experiment was carried out with 200 g. (3.3 mols) acetamide, 408 g. (4 mols) acetic anhydride and 20 g. (0.21 mols) acetamide hydrochloride in Quickfit apparatus.

Heat acetamide and acetamide hydrochloride in a 1.5 l. flask equipped with reflux condenser to about 130° (oil bath!) Add rapidly acetic anhydride which has been kept at 100° and continue heating. Boiling starts in 2 minutes and is allowed to continue for exactly 30 minutes. Atmospheric distillation between 100-140° lasts 45 minutes and yields 266 g. low boiling material. Further 134 g. of low boiling material are removed by distilling under vacuum (55-110°/35 mm). The main product distils sharp in vacuum: 94°/2.5 mm, 93°/2.0 mm, 92°/1.5 mm. The crude yield amounts to 209 g., m.p. 77-80°. The residue in the flask amounts to 7 g. and 2 g. of material are retained in the cold traps.

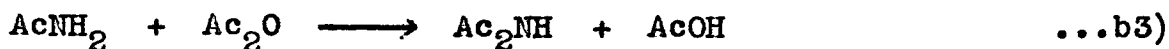
In 4 experiments (with minor variations) the yields were 202 g., 209 g., 214 g. and 202 g. In another experiment which gave a yield of 185 g. the preheated reactants were distilled without refluxing. For recrystallizing, a mixture of 150 cc. acetone and 300 cc. petroleum ether (60-80°) may be used for 200 g. crude product. The acetamide content of the crude material may be estimated by referring to the melting point curve of Dunn and Polya (23);

acetamide contents of 2-20% have been found in the crude products. About one recrystallization is needed to remove 6-7% acetamide. Thus three recrystallizations are needed for crude products containing 20% acetamide and one is sufficient when the acetamide content is 2% only. Loss on each recrystallization amounts to about 25%. The contents of the petroleum ether solutions from several experiments may be pooled and worked up as before.

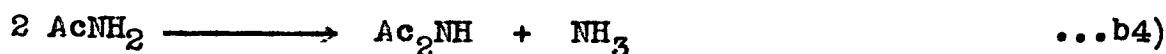
In the author's experiments using bisacetamide hydrochloride stopping atmospheric distillation at 125-128° and conducting the vacuum distillation at around 10 mm, crude yields vary between 240-280 g. with acetamide contents of traces to 12%. Two or three recrystallizations from 12 cc. petroleum ether (60-80°) per 1 g. crude product give 70-80% of the crude product as pure diacetimide, m.p. 79.5-80.5°_{corr.}, surface tension 67.8 dyne/cm at 18°, free from triacetimide. Organic Syntheses checkers worked on a similar method but purified the product by Strecker's process. They reported crude yields of 159-234 g. and pure yields of 114-156 g. However it appears that their vacuum distillation was not conducted with sufficient care since the crude products obtained by them melted between 45-54° in three experiments and 60-62° in one experiment. The eutectic point of the acetamide-diacetimide system is about 60° (23) and these results indicate the presence of lower boiling fractions in the crude product. The preheating of the reactants was omitted and it appears that the catalyst of the checkers lost hydrogen chloride through drying by inappropriate methods. (The catalyst loses its hydrogen chloride in vacuum or when stored over sulphuric acid, alkali and silica gel

The high boiling residues left in the distilling flask contain some acetamidine (probably as acetate) and an unknown base which has not been investigated in detail so far.

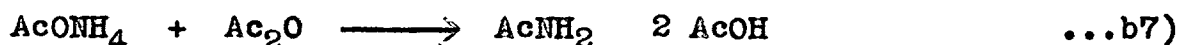
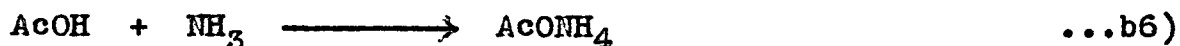
The acylation of acetamide by acetic anhydride was formulated by Hentschel as



The formation of acetic acid and diacetimide from acetamide and acetic anhydride does not prove this simple "mechanism". E.g. the following possibility could be considered:



The summation of equations b4) and b5) would give b3) and in this case the formation of diacetimide would be due to the condensation of acetamide and acylation of ammonia. Such views, however, are not plausible. It was thought unlikely that acetic anhydride would be much more effective than hydrogen chloride as a condensing agent. In fact hydrogen chloride in small amounts improves the yields of the Hentschel reaction which itself gives nearly three times the yield of diacetimide by condensation reactions without the possibility of acylation. The yield of the original Hentschel reaction is 50 parts of diacetimide from 100 parts of acetamide according to that author and somewhat less in our experience (1). With acid catalysts the yield rises to more than 100 parts of diacetimide from 100 parts of acetamide which excludes a pure condensation mechanism. It may be objected that even a pure condensation mechanism could furnish one mol of diacetimide from one mol of acetamide by the regeneration of acetamide from ammonia either according to equation b5) or

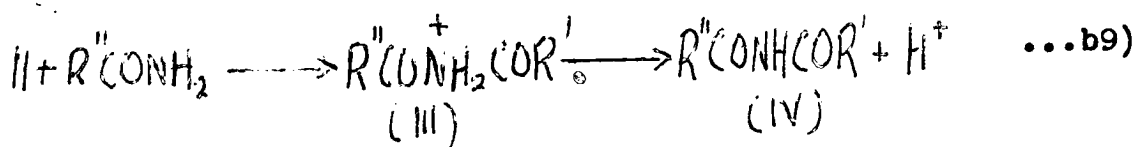
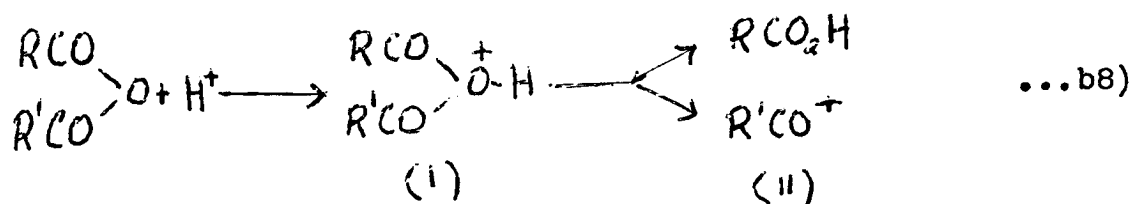


Reactions b5) and b6-7) probably occur to some extent but b6-7) is expected to be the more important reaction (since acetic acid reacts faster with ammonia than acetic anhydride) and it is known to occur rather slowly. Thus the formation of acetamide in quantities comparable with the original amount of reactant during the brief reaction time of the Hentschel reaction is unlikely. Acylation of acetamide may be the only reaction in the original Hentschel process. If the condensation reaction occurs at all the products resulting from it are undistinguishable from those of the acylation reaction. Small amounts of ammonium acetate have been found in distillation residues from the original Hentschel synthesis of diacetimide but the formation of ammonium acetate does not prove the occurrence of the condensation mechanism as it may originate from acetamidine acetate, which is known to be present. In the acid catalyzed Hentschel synthesis of diacetimide and similar syntheses of other amides ammonium chloride is formed. The collection of ammonium chloride was not always possible but in a number of cases 8-25% of the amount expected from the condensation mechanism were collected and weighed. There was no question of quantitative recoveries in any of these cases. The figures merely indicate the possible order of importance of the condensation reaction occurring simultaneously with the acylation. It was seen that in the pure condensation reactions yields of diacylimines decrease with an undue increase of the concentration of hydrogen chloride or acetyl chloride. This effect is not clear cut in the catalyzed Hentschel syntheses. Thus bisacetamide

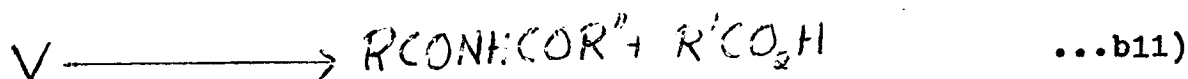
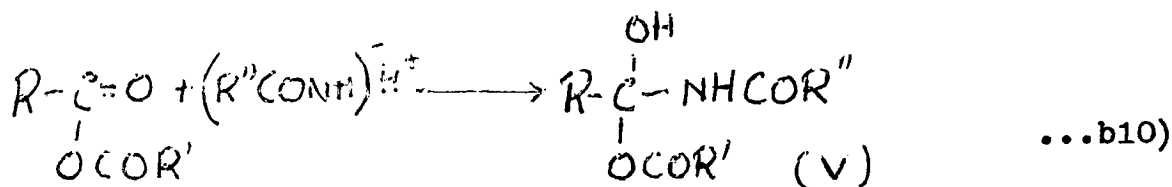
hydrochloride and acetamide hydrochloride when heated with a 30-33% excess of acetic anhydride give practically the same yields of diacetimide (1). The catalytic effect of acids on acylation was noted by Franchimont (82, 83) who, however, failed to apply it to the acylation of acetamide and relied on excess anhydride in his diacylimine preparations. This is rather surprising if one considers the following. Franchimont and Dubsky (84) noted that N.N'-dicarbethoxy-s-diaminoacetone cannot be acylated with acetic anhydride alone but the diacetyl compound is readily obtained if zinc chloride is used as a catalyst. Yet in the same paper these authors reported the tetra-acetylation of s-diaminoacetone with a large excess of acetic anhydride in the absence of catalysts. Next it will be remembered that Franchimont was the first to suggest that the mechanism of sulphuric acid catalysed acetylations involves the intermediate formation of acetyl sulphuric acid (85, 86). The formation of an addition intermediate in acid catalyzed acylations was confirmed by Smith and Orton (87, 88) who, like Skraup (89), Thiele (90) and Stillich (91), were inclined to interpret their results in accordance with Franchimont's views. This theory received some support from the work of Francis (92, 93) and Butler (94) on benzoyl nitrate which benzoylates amines. This analogy was not very satisfactory since Francis himself has shown (93) that benzoyl nitrate forms ethyl nitrate with ethanol, nitrates aromatic compounds (including phenols) and may contain 15-20% benzoic anhydride. While these early investigations may be regarded as the first instances of the fruitful ideas which have led recently to the establishment of the importance of compounds like acetyl phosphate in biochemistry

reactions (8, 9), the application of the concept of proton catalysis (95) have led opinions in a direction close to modern views on catalyzed acylations. The catalytic effect of pyridine on acylations by anhydrides (96) led Boeseken (97) to suggest that ionization (action dissociante) is the governing factor in catalyzed acylations. This, of course, 'is very similar to the notion of acid-base catalysis which was first formulated clearly by Lowry.

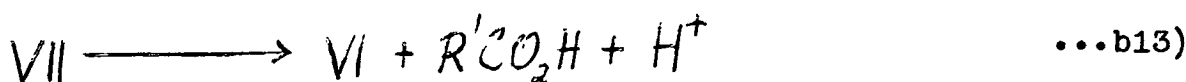
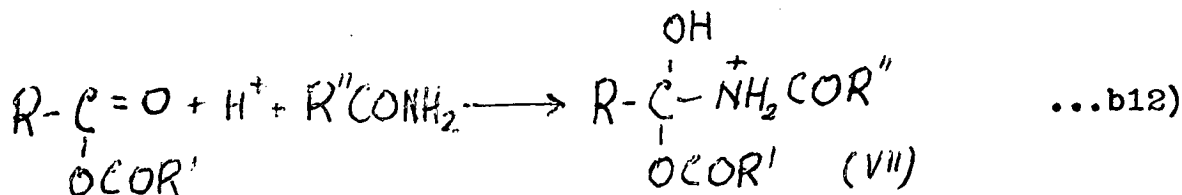
Designating the condensation reaction leading to diacylimines as reaction A and the genuine acylations as reactions B we shall proceed now to a discussion of the possible mechanisms involved in the original and modified Hentschel syntheses. The increase of yield in the acid catalyzed Hentschel synthesis is of a higher order than the extent of reaction A as indicated semi-quantitatively through the recovery of ammonium chloride. This suggests a B mechanism involving catalysed acylation. On recent views concerning acylations by anhydrides (98-103) the following mechanism (BI) may be considered in our case:



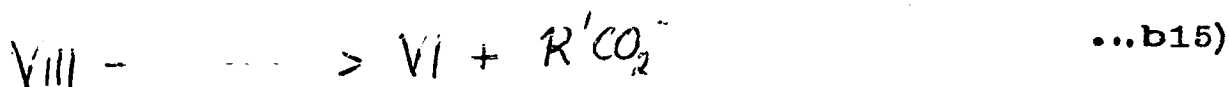
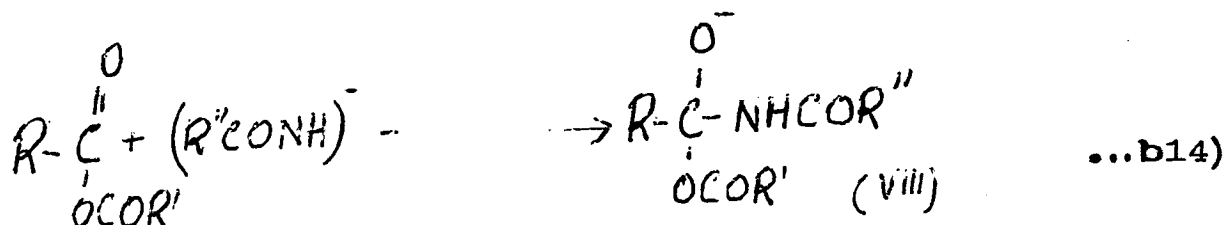
A second acylating mechanism (BII) must be postulated on considering that the original Hentschel reaction occurs with fair yield in the presence of acetic acid only and reaction A does not account for more than 0.5-1.0% of the yield judging from the amounts of ammonium acetate recovered after extracting the residues from the distillation with organic solvents. For formal reasons explained in a), such a reaction may be formulated on analogy with the hydrolysis of esters as an amidolysis of anhydrides:



This formulation approaches the view of Davies and Evans (104) on the hydrolysis of esters although it does not indicate whether an "acid" or "alkaline" process occurs. It may be considered that the reaction is initiated by the addition of proton or amidate anion:



or



Equations b12-13) are excluded by evidence showing that there is a mechanism BII such that it is not catalysed by acids. In favour of equation b14) one may point out that anhydrides are basic towards amides judging from titration experiments in chloroform with thymol blue as indicator (2) which justifies the assumption of the existence of amidate ions in the presence of anhydrides. Acylation of bases like aniline with acetamide is in agreement with these views. In view of the loose bonding between protons and anhydrides it is assumed that in reality there may be very little difference between the formulations b10-11) and b14-15).

Proofs for the suggested mechanism BI and BII are based on experiments in which R, R' and R'' were varied (2). The following predictions were made:

- 1) Mechanism BI will be catalysed by acids while BII will be inhibited in the presence of acids owing to the repression of the dissociation of the weakly acidic amide in the presence of a much stronger acid.
- ii) If R has a stronger Robinson-positive inductive effect than R', BI must lead to acylation by the weaker acyl radical, since acyl radical ions are formally similar

to protons.

- iii) In the same way BII will lead to acylation by the stronger acyl radical since the positive carbonium ion is more likely to be formed on that side through inductive effects.
- iv) In the absence of acid catalysts, amides with strong Robinson-positive R'' will contribute to BI. The same amides will react more readily by BII than less acidic amides.
- v) Strong Robinson-positive R or R' will inhibit the formation of I or VII but facilitate the formation of VIII. However the decomposition of I to II will be favoured and that of VIII to VI inhibited by the same effects.
- vi) The reactivities of anhydrides with high resonance energies will be small.

The verification of i) is rendered difficult by the fact that in acid catalysed reactions A, BI and BII may be superimposed. The average yields of diacylimines from reactions involving A only are of the order of 20% although acetamide and chloroacetamide in boiling xylene treated with a little dry hydrogen chloride afford a 37% yield of diacetimide. Ammonium chloride recoveries from acid catalysed reactions in the presence of anhydrides give similar values. Thus in the presence of acid catalysts the portion of yield amounting to about 20% (or more) of theory may be ascribed to mechanism A. In the absence of hydrogen chloride, acetamide with acetic anhydride give about 21% diacetimide; acetamide with propionic anhydride give 25% acetyl propionamide and chloroacetamide with propionic anhydride give 35%

chloroacetyl propionamide with negligible amounts of symmetrical diacylimines. Thus in the absence of hydrogen chloride the competition of A against BII is negligible. In reactions involving either A or BII alone the yields are approximately of the same order. In acid catalyzed reactions the increase of yields should be due to BI. Occasionally the yield of the acid catalysed acylation does not exceed greatly 40-50% (the approximate total of "isolated" yields from A and BII) which seems to indicate an inhibition of BII or A or both. The first of these possibilities is more likely since the semi-quantitative recoveries of ammonium chloride from such experiments do not appear to be correlated with variations of yield from one diacylimine to another.

Predictions ii) and iii) were tested by acylating acetamide, propionamide and benzamide with monochloroacetic anhydride, $\text{ClCH}_2\text{CO.O.COCH}_3$, in the presence and absence of hydrogen chloride. In the former case the yields were greatly increased (supporting i) and acetylated amides predominated over chloroacetylated ones in molar ratios of 1.5 for acetamide, 1.3 for propionamide and 1.6 for benzamide. In the absence of hydrogen chloride the ratios were inverted as follows: 0.75 for acetamide, 0.5 for propionamide and 0.8 for benzamide. Since no dipropionimide was formed in the acid catalysed reaction between propionamide and monochloroacetic anhydride it is assumed that little or no A reaction took place. The decrease of R'/R ratios in the absence of hydrogen chloride is greater for the more basic propionamide than for the more acidic acetamide and benzamide, in agreement with iv).

Acylations with monochloroacetic anhydride give lower yields than acylations with acetic or propionic anhydrides which

is in agreement with v) without completely proving this prediction. It is possible that when R and R' have inductive effects of opposite signs the resultant effect in the sense of v) is too small for measurement. On the other hand it was not possible so far to obtain conclusive results with mixed anhydrides with R and R' having inductive effects of the same sign but different magnitudes. Attempts were made to use acetic propionic anhydride (105) with closely similar R' and R by the author with the following results:

TABLE I

Products of acylation with acetic propionic anhydride

| <u>Amide</u> | <u>Catalyst</u> | <u>Products</u> |
|-----------------|-----------------|--|
| acetamide | HCl | 32% diacetimide 28% acetyl propionamide |
| propionamide | HCl | 29% dipropionimide 30% acetyl propionamide |
| chloroacetamide | HCl | 24% monochlorodiacetimide 17% propionyl chloroacetamide |
| benzamide | HCl | 22% acetyl benzamide 23% propionyl benzamide |
| acetamide | - | 11% diacetimide 13% acetyl propionamide |
| propionamide | - | 12% dipropionimide 12% acetyl propionamide |
| chloroacetamide | - | 7% monochlorodiacetimide 9% propionyl chloroacetamide |
| benzamide | - | 13% acetyl benzamide 15% propionyl benzamide |

The effects are similar to those observed by Polya and Spotswood for acylations with monochloro-acetic anhydride but the differences in R'/R ratios are hardly significant although greater differences would have been expected from the results of

hydrolytic and alcoholic experiments with acetic propionic anhydride (2, 105).

The fact that excess anhydride is necessary to obtain optimum yields (1, 81) may be interpreted in different ways but fits well the views of Burton and Preill (103) on acid catalysed acylations with acetic anhydride.

The failure of acylations with symm. dichloro-acetic anhydride in acid catalysed experiments (2) is in agreement with the first part of v). In later experiments of the author acylations with this anhydride were carried out in the absence of hydrogen chloride in an oil bath kept at 125-130° with a 300% excess of anhydride for 45 minutes. Using acetamide, propionamide chloroacetamide and benzamide the following compounds were obtained in low yields : monochlorodiacetamide (4%), propionyl chloroacetamide (5%), symm. dichlorodiacetamide (2%) and chloroacetyl benzamide (0.5%). Higher temperatures might have increased the yield but even under the milder conditions considerable charring occurred. These results are interpreted as inhibition of the formation of I necessary for the BI mechanism. The increased efficiency of the acylation in the absence of hydrogen chloride supports equations b14-15) as more likely than b12-13). On this view the formation of VIII would be enhanced but further reaction to VI inhibited. At the same time v) should be qualified by stressing that the argument concerns the anhydride rather than the amide in the reaction mixture. In the presence of a strongly acidic anhydride like the one under consideration the necessary dissociation of the amides would be repressed. Admittedly the relative yields and amidic acidities are not regularly

correlated but the difficulty of checking v) need no further excuse.

The low reactivity of benzoic anhydride with amides may be regarded as a confirmation of vi). In this case the absence of hydrogen chloride does not affect results. Yields of acetyl benzamide, propionyl benzamide and chloroacetyl benzamide obtained by the author under conditions similar to those described before the hydrogen chloride catalysed acylations of acetamide, propionamide and chloroacetamide with benzoic anhydride (2) are practically identical with the earlier results : 3%, 3% and 1% respectively. The effect of other acidic catalysts has been investigated by comparison with the experiments of Boeseken on the catalyzed acylation of urea (97). Since acetamide and urea have approximately the same molecular weight, the same quantities of amide and anhydride have been used as in Boeseken's experiments : 8.8 g. acetamide and 15 g. acetic anhydride. In Boeseken's experiments the reaction mixture was heated for one minute only but the acetamide reaction mixtures had to be heated for 30 minutes since heating for 1 minute gave low yields of diacetimide (of the order of 1-2%). The author's results are compared with those reported by Boeseken in Table 2.

TABLE 2.

| <u>Catalyst</u> | <u>Acetylurea</u> | <u>Diacetimide</u> |
|---|-------------------|--------------------|
| HClO ₃ (0.13 g.) | | 10.9 g. |
| AlCl ₃ (0.5 g.) | 12.7 g. | 2.2 g. |
| HCl (0.12 g.) | 11.1 g. | 10.2 g. |
| FeCl ₃ (0.62 g.) | 10.2 g. | 6.5 g. |
| H ₂ SO ₄ (0.4 g.) | 9.4 g. | 8.6 g. |
| ZnCl ₂ (0.5 g.) | | 7.3 g. |
| Cl ₃ COOH (0.4 g.) | | 5.1 g. |

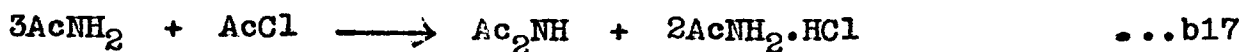
These experiments indicate the similarity between the acid catalyzed acylations of urea and acetamide. The only irregularity which may not be accounted for in spite of the presumably great inaccuracy of small scale synthetic methods is presented by the experiment on acetamide in the presence of aluminium trichloride. This is explained by the observation of P.L.Tardew in the author's laboratory that diacylimines form addition compounds with aluminium chloride. These addition compounds have not been examined closely so far but the experiments under discussion indicate that they decompose on heating with the formation of nitriles.

Acetyl chloride and other acyl halides catalyze the acylation of amides to diacylimines but the yields are somewhat lower than in the case of hydrogen chloride catalysis. From a theoretical point of view the discussion of the catalytic effects of organic acyl halides and thionyl chloride is impossible at present. Hydrogen chloride generated from such compounds may catalyze reactions A and BI. At the same time direct acylation may occur or even an indirect acylation through a Gautier

reaction. Competition experiments using acetic anhydride and acetyl chloride in equimolar amounts remained inconclusive. Apart from the fact that much nitrile was formed, particularly in the case of benzamide and the isomeric butyramides, the results could not be duplicated. Thionyl chloride is inferior to hydrogen chloride in the preparations of diacylimines. With acetamide : hydrogen chloride molar ratios of about 1 : 1, one part of diacetimide or more can be obtained from one part of acetamide whereas thionyl chloride in similar concentration gives acetonitrile as the main product with low yields of very impure diacetimide. With acetamide : thionyl chloride molar ratios of the order 10 - 20 : 1 yields of diacetimide approximate those obtained in hydrogen chloride experiments but there is still considerable nitrile formation and the product is of poor quality. In these experiments again duplication of yields from a number of runs was not possible. In general the author observed that whenever nitrile formation is considerable the acid catalyzed acylation of amides is difficult or impossible to control.

The acylation of amides with acyl halides instead of anhydrides has been used to prepare diacylimines by Titherley (25, 106), Dehn (107) and others (12, 108-110). Some experiments of Titherley and Dehn have been checked by Polya and Tardrew (1). Acetamide and acetyl chloride (equimolar mixture) in boiling benzene yields of 17-21 parts of diacetimide from 100 parts of acetamide (106). In boiling xylene the yield rises to 42%. The ammonium chloride isolated from the reaction corresponds to 34% diacetimide. Since the formation of ammonium chloride indicates mechanism A, it is seen that this mechanism is dominant over

mechanism B. When the reaction is carried out in boiling benzene little or no ammonium chloride is recovered, hence mechanism B is more important at lower temperatures. Dehn's reaction (in cold ether is inefficient for synthetic purposes.



One third of the available acetamide is acylated and the rest is withdrawn from the reaction as bis-acetamide hydrochloride. The reaction occurs nearly quantitatively according equation b17) and may be regarded as a pure case of mechanism B. The analogous reaction in benzene affords lower yields. From 100 parts of acetamide the ether reaction affords 34 parts of diacetimide and the benzene reaction 14 only. In ether the formation of the oxonium compound $(\text{Et}_2\text{O}.\text{Ac})\text{Cl}$ may be postulated. This makes a BI mechanism probable. Since no such intermediate may be considered in the case of benzene, a BII mechanism is more likely. These views are in good agreement with the fact that acetonitrile is formed in benzene but not in ether. It is also suggested that while the BII reaction with anhydrides involves predominantly the amide form the analogous reaction with acyl halides involves the imidol form. It may be of interest to point out in this connection that the reaction between acetamide and acetyl chloride in boiling xylene yields a small amount (0.55 g. per 100 g. acetamide) of a liquid boiling between $60-100^\circ/8$ mm. The original liquid does not solidify at room temperature but turns into a pasty solid at $5-10^\circ$. It is obviously different from acetamide or diacetimide. On keeping for a month or on heating on a waterbath for 4 hours it turns into diacetimide. It is free from chlorine and has a nitrogen content of $13.1\% \pm 0.1\%$. This excludes the "low-melting

variety" of triacetimide (32). The most plausible explanation of the structure of this substance is to regard it as a somewhat impure isomer of diacetimide, probably $\text{CH}_3\text{C}(\text{OAc})\text{:NH}$. This hypothesis is confirmed by the exceptional ease of hydrolysis. A 10% solution in cold water deposits droplets of acetic anhydride in 15-20 minutes. Boiling the solution for 15 minutes gives ammonium acetate. Attention was drawn earlier to the lowered stability of diacetimide purified by Strecker's method. It is possible that high acid concentration effects a change to "isodiacetimide" although the isomerization was never observed to occur to a significant extent. This observation raises again the question of isoamides or iminohydrins which was thought to have been disposed of by Rule (111). In the case of our compound ready isomerization on standing at room temperature excludes the possibility of acetyl acetamidine acetate and the approximately correct nitrogen analysis shows that the compound is not acetyl acetamidine (related to imidodibenzamide (61) which melts much lower than dibenzamide).

The mechanism of the acylation of amides by acyl halides has been investigated by Titherley and Holden (25, 26). Their results suggest that acyl halides may react either with the amide or imidol form of the other reactant. In the latter case diacylimine formation is increased. This occurs when there is a great excess of amide over acyl halide which is explained by Titherley and Holden by the already discussed formation of amide hydrochlorides and the assumption that free hydrogen chloride catalyzes the transition of the acidic imidol form into the basic amide form. The failure of the benzoylation of acetamide by benzoyl chloride (106) is explained

then by assuming a preponderantly amide character in acetamide. The author has come to this conclusion on other, somewhat similar, arguments. Physical evidence for this view will be quoted in a later chapter.

From a practical point of view acylation of benzamide with aliphatic acyl rests is conveniently accomplished by treating benzamide with aliphatic acyl halides. This seems to be the only practicable method for the preparation of chloroacetyl benzamide (2). *n*-Butyramide and isobutyramide may be benzoylated with benzoyl chloride. This is in conformity with Titherley's views on the requirement of the imidol form for this reaction and with the author's views on the enhanced imidol character of these amides. Butyryl benzamide has been prepared before through the butyrylation of benzimino methyl ether (112). Its new preparation is given below for comparison with the preparation of isobutyryl benzamide which is a new compound.

n-Butyryl benzamide.

Benzamide (12.1 g.), *n*-butyric anhydride (19.0 g.) and *n*-butyryl chloride (0.4 g.) prepared from butyric acid and thionyl chloride by repeated treatment and evaporation with vapours of petroleum ether (60-80°) were reacted as in the preceding experiment. Very little cloudiness and no distinct precipitation occurred during the reaction. No measurable amount of ammonium chloride could be isolated. The crude product did not crystallize on cooling. It was distilled under atmospheric pressure yielding 23 cc. distillate which contained mainly *n*-butyric acid, some *n*-butyric anhydride and a trace of benzoyl chloride. The latter indicates some exchange of functions between the benzoyl

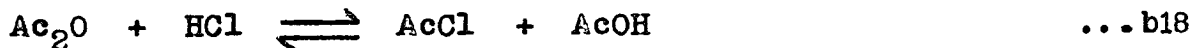
and n-butyryl rests and will be investigated in detail. The residue from the distillation crystallized on keeping in the refrigerator. The crystals were filtered by suction and recrystallized 3 times from 40 cc. petroleum ether (60-80°). This reduced the yield considerably but less purified material was discoloured even after treatment with Norite and gave too high figure for nitrogen. Altogether 3.1 g. pure material have been obtained (16% on assuming a pure B mechanism of acylation). The substance is microcrystalline. Needles are obtained on dissolving the pure material in a great excess of petroleum ether and allowing the solvent to evaporate at room temperature. The melting point is 68-69°. N calculated for n-butyryl benzamide 7.33%, found 7.18% (P.D.). On further crystallizations from hot water and finally from petroleum ether the melting point rose to 104°; N found 7.32%. The great difference between the melting points for an analytical difference close to the order of experimental error is surprising. It is likely that the low and high melting materials represent isomeric forms of n-butyryl benzamide. Neither of these isomers, if isomerism occurs in this case, could be an iminoester, $RC(OOCR'):NH$ since both materials can be recrystallized repeatedly from hot water without a substantial change in nitrogen values. Both the low and high melting material gave calculated acid equivalents. On hydrolysing 0.1 - 0.4 g. amounts of both materials with 2-8 cc. $M/1$ NaOH, concentrating to one fifth of the volume on a water bath and acidification with the minimum amount of hydrochloric acid with external ice cooling benzoic acid separated. It was collected on a micro-filter, washed with a little ice water, dissolved in ether, washed with saturated sodium chloride solution,

freed from solvent and assayed by titration with N/100 sodium hydroxide. In each case 91-94% of the expected amount of benzoic acid could be recovered. The loss of benzoic acid corresponded to the solubility of benzoic acid in the given volume of the aqueous phase.

Isobutyryl benzamide.

Benzoyl chloride (12.5 cc.) and isobutyramide (4.35 g.) were reacted as in the preceding experiments. Ammonium chloride appeared as in the preparation of acetyl n-butyramide (about 80 mg. or 3%). On cooling the hot filtered reaction product by immersion in water, crystals were obtained which were filtered and washed with petroleum ether (80-100°). On combining the petroleum ether washings with the oil from the crystals a little more crystalline material was obtained. Distillation of the filtrate from the second crop of crystals gave isobutyric acid. Since decomposition started on attempting to distil the excess benzoyl chloride under atmospheric pressure the distillation was interrupted and the residue was repeatedly crystallized from petroleum ether (60-80°). Little material was obtained in this way and the product retained some benzoyl chloride. It was taken up in ether, washed 3 times with ice-cold N/10 sodium hydroxide, 3 times with ice-cold water, dried and freed from the solvent. The product was combined with the first crop of crystals and recrystallized once from boiling water with Norite and once from 50 cc. petroleum ether (60-80°). Fine needles were obtained in a yield of 0.7 g. (7%); melting point 121°; mixed melting points with isobutyramide and benzoic acid were depressed by up to 60°. Nitrogen required for isobutyryl benzamide 7.33%, found 7.23% and 7.28%.

Acylation by acyl halides are of some importance in the acid catalyzed acylations by anhydrides. The equilibrium



was studied by Brooke (113). Titherley (25) may have underestimated the importance of this equilibrium since ^{he}was not concerned with acid catalyzed acylations of amides and considered acylations by anhydrides rather unsatisfactory (24). It appears, however, on reviewing Titherley's and the author's experience that acylations with anhydrides and acyl halides vary in efficiency from amide to amide. In the case of acetamide (with amide character) acylation with a catalytic amount of hydrogen chloride and acetic anhydride gives good results. With an increase of the hydrogen chloride concentration acetyl chloride is formed which depresses the efficiency of the reaction particularly as the large concentration of hydrogen chloride tends to preserve the amide form. In the case of benzamide (with imidol character) acylation with acyl chlorides gives excellent yields but yields from hydrogen chloride catalyzed acylations with anhydrides are depressed owing to partial conversion to the basic amide form (26) which reduces the concentration of the catalyst required for a BI reaction. In the case of amides of an intermediate character, like n-butyramide, neither method is quite satisfactory as illustrated by the following preparations. N-dibutyrimide has been prepared by other authors using different method (72, 114).

Acetyl n-butyramide.

n-Butyramide (8.7 g.), acetic anhydride (11.0 g.) and acetyl chloride (0.5 cc.) were mixed and refluxed for 40 minutes. After a few minute's heating the mixture became cloudy and a white

precipitate appeared. On further heating the quantity of the precipitate did not seem to increase. The mixture was filtered hot on a Buchner funnel. The precipitate was washed with a little cold ether and the filtrate was kept separate from the filtered reaction product. The precipitate was identified as ammonium chloride (0.34 g. or 6.4%). The reaction product crystallised rapidly on cooling. The crystals were filtered and washed with petroleum ether (80-100°). The filtrate from the crystals and the petroleum ether washings were united. More crystalline material separated and was filtered. The filtrate was distilled under atmospheric pressure. Most of the distillate came over between 90-100° and very little distillate was collected between 110-120° (most of the acetic acid formed in the reaction distilled with with the vapours of the petroleum ether). The residue in the flask was washed with fresh petroleum ether, the resulting crystals were filtered and the filtrate was distilled as before. Treatment of the residue with petroleum ether afforded little crystalline material. The crystals were filtered off and the filtrate was discarded. The second and third crop of crystals were joined and purified separately from the first crop using the same method in both cases. The crystals were recrystallized from boiling water. After recrystallization the first crop melted unsharply between 101° and 105° and the combined later crops between 95° and 100°. A mixed melting point of 96-102° was observed. The united crystals were recrystallized from boiling water and gave material melting at 115-116°. The crude yield at this stage amounted to 6.1 g. Since about 200 cc. water has been used in the recrystallizations and since the solubility of n-butyramide

in cold water is 3.7 g. in 100 cc., the bulk of any unchanged n-butyramide must have been eliminated at this stage. A final recrystallization from 120 cc. petroleum ether (60-80°) gave 5.8 g. of fine white needles melting sharply at 118°. On the spontaneous evaporation of the mother liquor another 0.1 g. of material was obtained in the form of large, thick needles melting unsharply between 70° and 80°. The bulk of crude material and the thick needles were combined and recrystallized from petroleum ether. The final product weighed 5.8 g. (45% on assuming a B mechanism only) and melted at 119°. Mixed melting points with approximately equal amounts of n-butyramide ranged from 60° to 77°. On alkaline hydrolysis 0.1342 g. acetyl n-butyramide require 20.9 cc. N/10 NaOH, found 20.9 cc; N calculated 10.84%, found 10.79% and 10.81%. The hydrolysate contains both acetate and n-butyrate. On heating 0.129 g. of the substance with 0.093 g pure aniline for 60 minutes, dissolving the product in 100 cc. ether, washing with ice cold N/10 hydrochloric acid to remove unreacted aniline and removing the solvent 0.135 g. material have been obtained. After washing 3 times with petroleum ether (80-100°) of 70-75° in order to remove any unreacted diacylimine 0.131 g. material was obtained and analysed for nitrogen. From the value of 8.4% N it was calculated that the product contained 72% acetanilide and 28% n-butyranilide.

n-Dibutyrimide (with P.D.).

n-Butyramide (4 g.) was dissolved in dry ether and treated with dry hydrogen chloride for 30 minutes. The precipitate of butyramide hydrochloride, $\text{PrCONH}_2 \cdot \text{HCl}$, was filtered, washed with ether and dried quickly by suction just before use.

n-Butyramide (10 g.) butyramide hydrochloride (2 g.) and n-butyric anhydride (22 g.) were refluxed for 40 minutes. The low boiling fractions were removed by distillation at $80^{\circ}/35$ mm. The residue crystallized on cooling. It was washed with cold water and recrystallized from hot water. The pure product (1.6 g., 8%) melted at 111° , a few degrees higher than the products reported by Tarbouriech and Miller (107° and 108°) respectively.

The same product in somewhat better yield has been obtained by the author with the following modifications. Butyramide hydrochloride was replaced by 0.4 g. n-butyryl chloride and the product was crystallized from petroleum ether ($60-80^{\circ}$) instead of hot water. In this way the same product was obtained in a yield of 2.1 g. (11%).

The following experiments have been carried out to investigate the scope of acid catalyzed acylations for the preparation of N-substituted diacylimines and a few N,N-disubstituted amides. This method seems to have been originated by Musselius (116). It did not become popular and most of the reported syntheses of N-substituted diacylimines are based on other methods. The direct acylation of aniline with acetic anhydride gives a mixture of acetanilide and diacetanilide, the latter in 52% yield (117). The acylation of phenyl mustard oil (76) gives about the same yield. The acid catalyzed acylation gives somewhat higher yields and it is more convenient than the acylation of phenyl mustard oil. The yields are much lower in the aliphatic series. N-methyl and N-ethyl diacetimide (28, 33, 75, 81, 107, 118) have been prepared in the author's laboratory with P. Dunn by heating

methylamine hydrochloride or ethylamine hydrochloride with acetic anhydride and fractionating the reaction products. The yields were 40% and 24% respectively. The low yields were due mainly to considerable losses on fractionation which make small scale preparations very wasteful. If preparations were carried out on a large scale continuously or on a large number of occasions recoveries from fractions discarded in our small scale experiments would at least double the yields, judging from the boiling range of such fractions. These and other experiments on acid catalyzed acylations leading to N-substituted diacylimines and N,N'-disubstituted amides will be given in more detail and supplemented with further experiments, with which the author has not been concerned personally, in a M.Sc. Thesis which is being prepared by P. Dunn. As regards N-methyl diacetimide it will be enough to note the following at present. This compound is soluble in ether,,contrary to the statement of Hentschel (81) although the analysis and boiling point were identical in Hentschel's experiments and ours. When heated with aniline N-methyl diacetimide gives a quantitative yield of acetanilide free from N-methyl acetanilide. The preparation of N-methyl dipropionimide, a new compound, will be given below.

N,N-dimethyl and N,N-diethyl acetamide (80, 116, 119) have been prepared from dimethylamine hydrochloride and diethylamine hydrochloride and acetic anhydride in yields of 71% and 41% respectively. The products contained some N-alkyl acetamide.

Unsymmetrical N-aryl diacylimines have been prepared by Wheeler and his collaborators (120-122) by acylating silver or mercury acylanilides. It was found that this somewhat incon-

venient method may be avoided by acid catalyzed acylation of acetanilide for the preparation of propionyl and butyryl acetanilide. In the case of benzoyl acetanilide, however, the acid catalyzed acylation failed.

N-methyl dipropionimide (with P.D.).

Methylamine hydrochloride (11 g.) and propionic anhydride 60 cc. were refluxed for 6 1/2 hours. In a first distillation all material boiling between 180-212° was collected. In a second distillation all the fraction boiling between 202-212° (mostly between 205-212°) was kept and redistilled at 209-212°.

Yield : 4.0 g. (17%); N calculated 9.80%, found 9.98%; the Lipmann-Tuttle test was positive. The substance is very soluble in water, alcohol, ether, chloroform but insoluble in hydrocarbon solvents. With aniline it gives propionanilide. Like N-methyl and N-ethyl diacetimide, it is hygroscopic and deteriorates rapidly on standing if not well protected from moisture.

Diacetanilide (with P.D.)

Acetanilide (34 g.), acetic anhydride (102 cc.) and acetyl chloride (5 cc.) were refluxed for 12 1/2 hours. Lower boiling fractions were removed by distillation under atmospheric pressure up to 180°. The resinous residue was dissolved in 5 times its weight of benzene, boiled with Norite, filtered, freed from solvent and cooled in a freezing mixture. The solid was rapidly filtered and washed with a little cold ligroin to give a crude product of 42 g. Digestion with 750 cc. ligroin at 30-40° dissolved the diacetanilide. On freezing it separated and was purified by repeating this treatment. Yield : 27 g. (60%); m.p. 36°; N found 7.93%, calculated 7.90%. No p³-acetylamino-

acetophenone could be detected in the reaction products although the conditions of the reaction approximated those of Chattaway (123) who converted diacetanilide into the former compound by heating with hydrogen chloride. Franzen (124) described the preparation of acetanilide from aniline hydrochloride and acetic anhydride but did not state the yield nor did he investigate the presence of diacetanilide.

Acetyl propionanilide (with P.D.)

Acetanilide (30 g.), propionic anhydride (50 cc.) and thionyl chloride (5 cc.) were refluxed for 12 hours. On cooling the reaction mixture set to a thick brown paste. On shaking with 1000 cc. cold petroleum ether (80-100°) crystals were formed. These were filtered (13 g.) dissolved in ether, twice decolorized with Norite, freed from solvent and twice recrystallized from boiling petroleum ether. The resulting material melted unsharply at 95-98°. On further recrystallization from boiling water the melting point rose to 103-104°. The material was identified as propionanilide; N found 9.27%, calculated 9.38%; yield 9.5 g. (30%). The filtrate from the crude crystals of propionanilide was freed from petroleum ether in vacuum. The residue was distilled in vacuum; the fraction boiling between 160° and 165°/15 mm. was redistilled at 162-164°/15 mm. and afforded 17.6 g. (41%) acetyl propionanilide; N found 7.27%, calculated 7.33%.

Acetyl butyranilide (with P.D.)

Acetanilide (30 g.), n-butyric anhydride (80 cc.) and thionyl chloride (3 cc.) were reacted and worked up as in the preceding experiment. After distilling off fractions boiling below 180° under atmospheric pressure the residue was distilled in vacuum

collecting the fraction boiling between 140° and $170^{\circ}/10$ mm. Redistillation ($168-172^{\circ}/14$ mm) gave the desired product which had a similar boiling range on redistillation. Yield : 16.7 g. (36.5%). No butyranilide could be isolated from the various fractions.

N,N-diacetyl 1-naphthylamine (with P.D.: cf. 117, 125, 126)

1-Naphthylamine (30 g.), acetic anhydride (80 cc.) and acetyl chloride (10 cc.) were refluxed for 10 hours. On being allowed to stand at room temperature for 2 days crystals separated and were filtered, washed with two 200 cc. lots of water and recrystallized from 350 cc. hot ethanol. The precipitation of the material was completed by adding 300 cc. water. The oily filtrate from the first crystallization was shaken with water. The oily crystals were filtered and recrystallized from aqueous alcohol as before. The same material, N,N-diacetyl 1-naphthylamine was obtained in both cases. The combined yield was 36 g. (76%); m.p. 128° ; N found 6.10%, calculated 6.17%. The aqueous ethanol mother liquors were diluted with more water heated to 80° , and cooled in a refrigerator over night. Crystals were collected and recrystallized from 4 200 cc. lots of petroleum ether ($80-100^{\circ}$). In this way 1 g. (3%) N-acetyl 1-naphthylamine, m.p. $149-150^{\circ}$ was obtained.

N, N-diacetyl 2-naphthylamine (with P.D.,; cf. 117)

Experiments similar to the previous one gave poor results. The following method was found satisfactory. 2-Naphthylamine (30 g.) acetic anhydride (70 cc.) and acetyl chloride (10 cc.) were refluxed for 12 hours. Low boiling fractions were removed up to $80^{\circ}/18$ mm. The black residue was dissolved in 200 cc. ethanol,

decolorized with Norite, filtered and freed from solvent. The residue was extracted 5 times with 200 cc. lots of petroleum ether (80-100°). After decolorizing with Norite and cooling a small amount of crystals (4 g.) were obtained. The petroleum ether was slowly distilled. When only about 100 cc. were left in the flask a yellow oily layer separated. On cooling it set to yellow, resinous crystals (22 g.). The combined crystals were extracted with 200 cc. ether in the cold, leaving a small amount of white material undissolved. Treatment with Norite and evaporation of the solvent left 20 g. oil (43%) which solidified to yellow-white crystals, (m.p. 67°) on cooling. N. found 6.15%, calculated 6.17%.

N-acetyl diphenylamine (with P.D.; cf. 127, 128).

Diphenylamine (20 g.), acetic anhydride (60 cc.) and acetyl chloride (5 cc.) were refluxed for 3 hours. Distillation under atmospheric pressure up to 150° removed lower boiling fractions. The residue solidified on cooling. It was extracted with 200 cc. boiling petroleum ether (70-90°) and filtered rapidly while hot. On cooling crystals on m.p. 99-100.5° are obtained in a yield of 17.7 g. (71%); N found 6.39%, calculated 6.63%. Obviously the material was not quite pure but since it has been described before and we were interested in the practicability of its synthesis by acid catalyzed acylation no further attempts were made to purify it by more recrystallizations from petroleum ether.

From an industrial point of view it would be interesting to test the acylation of amides with acyl halides under pressure. Reactions of this kind have been carried out by Tarbouriech (114, 115) who, however did not investigate the effects of varying

the pressure.

Acylations of formamide by acetic and propionic anhydride give unidentified high boiling substances and 28% acetamide or propionamide (2). The author obtained 26% n-butyramide, 35% isobutyramide and 19% benzamide in similar reactions of formamide with n-butyric, isobutyric and benzoic anhydrides. Formyl acetamide has been prepared by the acylation of formimino ethyl ether hydrochloride with acetic anhydride in the presence of sodium acetate (129). This method is of general use in the preparation of diacylimines (130-135) in addition to the similar method of acylating amidines (136-139).

Acylations discussed thus far referred to acidic conditions. Attempts to carry out amide acylations under conditions similar to those of the Schotten-Baumann reaction failed (140). Satisfactory acylation of amides with acyl halides in pyridine has been reported by Titherley (24) and Freundler (141-143), except that the use of the more reactive acyl halides in this reaction requires low temperatures to prevent addition to pyridine. Experiments on base catalyzed acylations of amides are in progress in the author's laboratory. It is preferred, however, to withhold the available results for the time being since neither the analytical nor the synthetic work have been sufficiently checked. Aqueous alkali or alcoholic sodium alkoxides cannot be used, of course, since they lead to rapid hydrolysis or alcoholysis. A two-step reaction which amounts to acylation in a basic medium is the acylation of the alkali derivatives of amides with acyl halides or anhydrides which has been studied by Titherley (37) and Blacher (144, 145). Sodium benzamide is readily acylated to

acetyl benzamide by acetyl chloride but the acylation of sodium acetamide with benzoyl chloride yields dibenzamide in addition to benzamide, tribenzamide, phenyl cyanide and benzoic anhydride (34). The intermediate formation of acetyl benzamide cannot be assumed in this reaction since it is stable under the mild experimental conditions (refluxing in benzene). A possible interpretation in agreement with previously voiced theories, the stabilization of the imidol form of benzamide through the formation of the sodium compound, explains one half of the data only. A similar stabilization should occur with acetamide as suggested by the eventual transfer of the nitrogen from sodium acetamide to the benzoyl rest. Since the aliphatic sodium imidolate does not add benzoyl chloride, it is assumed that the energetically less probable imidol form is unstable in the case of acetamide while it is stabilized by resonance when attached to an aromatic nucleus. Both sodium benzamide with acetic anhydride and sodium acetamide with benzoic anhydride give sodium dibenzamide (145). Benzoic anhydride, usually a poor acylating agent for amides, appears to be very effective in this reaction. These reactions present many unsolved problems and their extension to cover a wider range of amides, anhydrides and acyl halides deserves resuming interest in this field, which has been neglected since Titherley's time.

A few experiments were carried out on the acetylation of amides with ketene. A ketene generator was built and operated by Mr. D. Padgham in the course of his M.Sc. work under the direction of M.J.E.Cran. The generator was similar to that described by Morey (196) and was calibrated with n-butanol. The

amides were fused and kept at temperatures 10-15° above the melting points of the highest melting product expected to be found in the reaction mixture. Although a large excess of ketene was used in some experiments the yields were low and much unchanged ketene was trapped in aniline. It appears that the amides are not very reactive in this reaction although better results would have been obtained had it been possible to use larger quantities of amides and thus secure more effective contact with ketene than that afforded by a few grams of amide in a test-tube of 1" diameter. The crude reaction products have been worked up by the author. The results indicate that the reaction may be of eventual interest but the first problems to be considered are in the field of chemical engineering and will be left to Mr. D. Padgham who indicated his interest in continuing this work during 1951. Experiments which gave positive results are summarized in Table 3. The methods of separating amides and discylimines have been described before in this Thesis and other publications and need not be repeated. Yields are calculated from the amides.

TABLE 3.

| <u>Reactants</u> | | <u>Recovered</u> <u>Amide %</u> | <u>Other Products</u> <u>(% yields)</u> |
|---------------------------|----------------------|------------------------------------|--|
| <u>Amide (mols)</u> | <u>Ketene (mols)</u> | | |
| AcNH ₂ , 0.2 | 0.2 | 66 | Ac ₂ NH 21; trace MeCN |
| AcNH ₂ , 0.03 | 0.2 | 29 | Ac ₂ NH 56 (see below) |
| Ac ₂ NH, 0.1 | 0.2 | 72 | Ac ₃ N 11; resin(0.2 g) |
| PhCONH ₂ , 0.1 | 0.1 | 52 | PhCONHAc 17; PhCN 17 |

The diacetimide from the experiment with acetamide and a large excess of ketene is obtained in the form of an oil which is incompletely soluble in water and deposits of droplets of acetic

anhydride. The ethereal solution of this oil reacts with sodium to give a yellow pasty solid which gradually changes into white granules of sodium diacetimide. The nitrogen content of the oil is approximately correct for diacetimide (N found 14.17%, calculated 13.86%). After standing for a few hours at room temperature in a sealed tube the oil begins to crystallize. After 18 hours transformation to diacetimide, m.p. $78-9^{\circ}$, is complete.

In the reaction of diacetimide with ketene a transitory green colour appears within a few minutes and turns dark after 15-20 minutes' exposure to ketene. No similar colour reaction was observed with acetamide.

The reaction between n-butyramide (0.1 mol) and ketene (0.25 mol) was unsuccessful. The crude product contained the anhydride of n-butyric and acetic acids, unchanged n-butyramide and only a few milligrams of what appeared to be impure acetyl n-butyramide.

Formamide reacted vigorously with ketene. The crude product contained 43% unchanged formamide, traces of hydrogen cyanide, 16% of acetic acid (with formic acid calculated as acetic acid) and an unidentified oil. The oil is soluble in ether but little soluble in petroleum ether. The contents of the petroleum ether (60-80^o) solution were recrystallized from ether by slow evaporation and yielded 0.12% formyl acetamide, m.p. 70° (128). The residual oil is being investigated at the time of writing.

At the present stage of our investigations the acetylation of amides by ketene does not appear to be a useful synthetic method except for the preparation of the unstable isomer of diacetimide.

c) Addition of acids and acyl halides to nitriles.

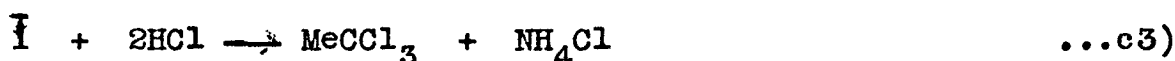
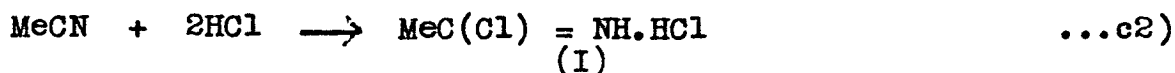
Gautier (22) obtained diacetimide by heating acetonitrile with acetic acid under pressure. In the absence of pressure the reaction



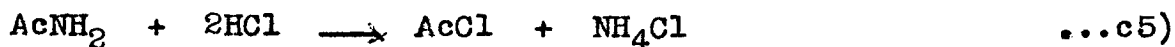
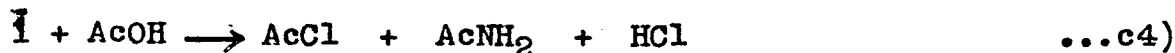
is displaced to the left at higher temperatures (81). In the author's experiments prolonged refluxing of acetic acid and acetonitrile (both highly purified and sharply dried) for 48 hours with the exclusion of atmospheric moisture did not yield any diacetimide. On repeating the experiments in the presence of a trace of dry hydrogen chloride small amounts of diacetimide (or triacetimide) were formed. M/4 experiments afforded too little diacetimide (or triacetimide) for isolation and purification. The formation of diacetimide or triacetimide was ascertained by the hydroxamic acid reaction. In other experiments a stream of dry hydrogen chloride was passed through a refluxing mixture of equimolar amounts of acetonitrile and acetic acid for 50 hours. These experiments were interrupted at night and the mixture was allowed to stand in a sealed apparatus under an atmosphere of hydrogen chloride. 62-75% of the nitrogen was recovered in the form of ammonium chloride which precipitated gradually during the reaction. Removal of the lower boiling fractions followed by the usual procedure afforded diacetimide in yields of 3-4%. Careful neutralization of the lower boiling fractions with aqueous sodium bicarbonate and extraction with ether gave an oil containing nitrogen and chlorine. This was hydrolyzed with N/2 sulphuric acid, neutralized with sodium carbonate and extracted with ether.

On removing the solvent in vacuum 1, 1, 1-trichloroethane was

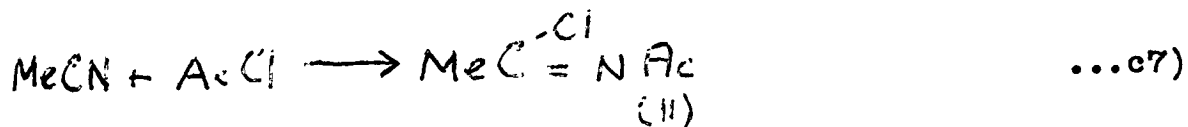
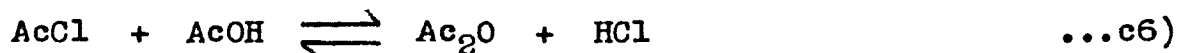
obtained in addition to some unchanged acetonitrile. On repeating the process for the removal of acetonitrile almost pure, 1,1,1-trichloroethane was obtained in yields of 4-6%; b.p. 75-76°; Cl found 79.61%, calculated 79.94%. The low yield of 1,1,1-trichloroethane in comparison with the high yield of ammonium chloride suggests that the reactions



are less important than others for the formation of ammonium chloride. If we assume that all the diacetimide is formed through the Gautier reaction, the greater part of the ammonium chloride remains unaccounted for. The sequence of reactions



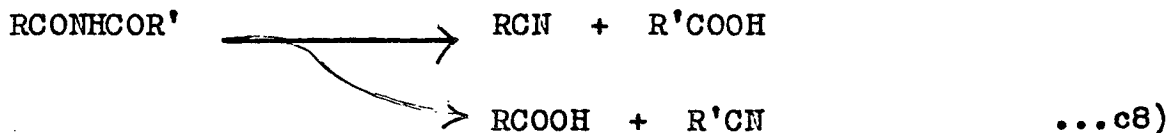
could explain the presence of large amounts of ammonium chloride but would require further reactions



to fit the observation that acetyl chloride occurs in traces only in the reaction product. Admittedly neither of reactions c6) and c7) is plausible. The equilibrium of reaction c6) would be displaced to the left under the experimental conditions and II should afford diacetimide on treatment with aqueous sodium bicarbonate solution in a yield of the same order as that of ammonium chloride.

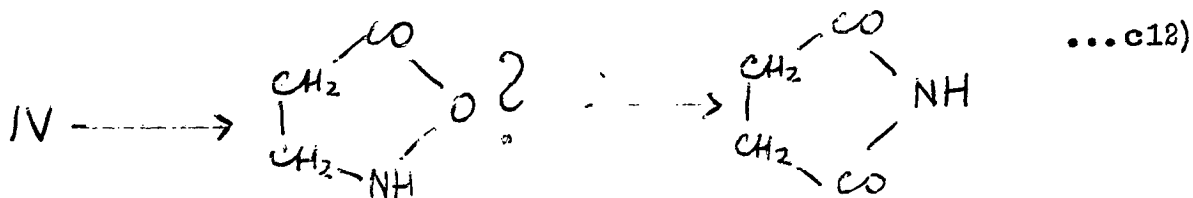
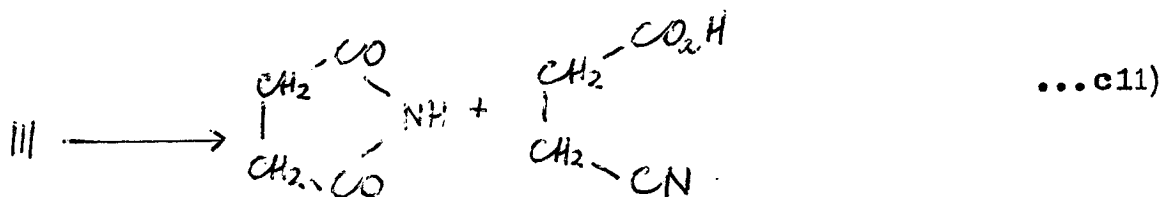
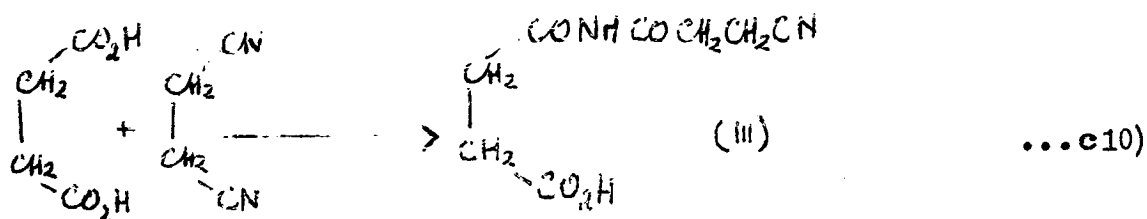
It is possible that II is comparatively stable in cold aqueous sodium bicarbonate solutions for short times of contact and that it is decomposed only during hydrolysis with dilute sulphuric acid. Attempts to isolate the hypothetical compound II have failed so far.

The reaction between acids and nitriles was investigated in detail by Colby and Dodge (146) who found the process satisfactory for systems of aliphatic acids with aliphatic or aromatic nitriles. In systems of aromatic acids and aliphatic nitriles there is an exchange of functional groups. Wholly aromatic systems give the expected diacylimines in addition to other products. These results were confirmed and extended by König (147) who obtained a number of halogenated diacetimides in good yield by this method. König also proved the until then debated "symmetrical structure" of diacylimines by obtaining identical products, formulated as $RCONHCOR'$, from the systems $RCN-R'COOH$ and $RCOOH - R'CN$. For further examples of this method see (11, 114, 148-151). The reaction in this form has many puzzling features. The best yields are obtained with halogenated aliphatic acids if care is taken not to work at high temperatures at which halogenated diacylimines tend to dissociate ^{and} rearrange;



This would be in agreement with the fact that addition to nitriles usually requires acid catalysis. However König's synthesis of

1,1,1-trichlorodiacetamide from acetonitrile and trichloroacetic acid failed while the weaker acetic acid and monochloroacetic acid reacted with acetonitrile. The failure of thioacetic acid to react with nitriles may be due to secondary reactions which have not been investigated so far. Phenyl cyanide and phenyl acetic acid did not react in König's reaction although they react with other acids and nitriles respectively. Similarly the author (with T.M.S.) found that the unknown dihomoveratroimide cannot be prepared by reacting homoveratroic acid and homoveratronitrile under König's conditions. Succinic acid and succinonitrile react to succinimide (147), possibly through the following mechanism (cf. 148)

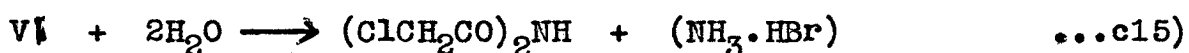
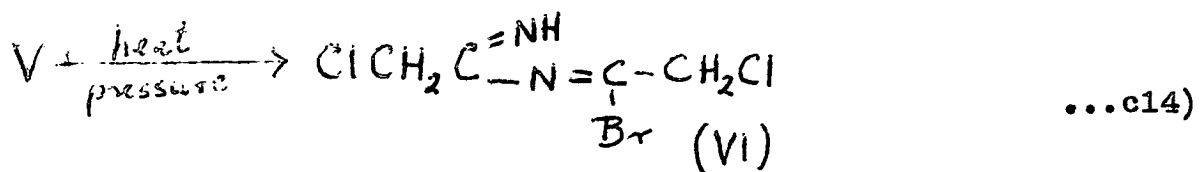
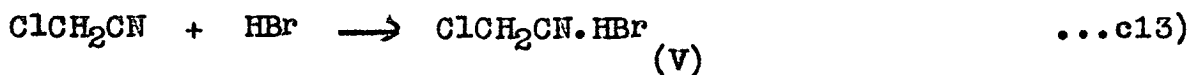


An interesting but little investigated variation of this method is the n-divalericimide synthesis of Lieben and Rossi (152) who reacted n-butyl chloride with potassium cyanide in 85% ethanol under heat and pressure.

The formation of diacylimines from nitriles and acyl halides on heating under pressure has been studied in some cases. Attention was drawn before to the possibility that a reaction of this nature occurs during the acylation of amides by acyl halides or even during acid catalyzed acylation of amides by anhydrides. It is known that amides may be converted into anhydrides on heating with acetic anhydride (153), acetyl chloride (140), thionyl chloride (154), aluminium chloride (155) and other metallic salts (156,157), to mention a few examples only which refer to experimental work quoted before. In the experiments of Kremann, Zoff and Oswald (158-160) on an equimolar mixture of benzamide and acetic anhydride the molar ratio of acetyl benzamide and phenyl cyanide was found to be approximately 3:1. That the reaction between nitriles and acids is not necessarily a major contribution to the formation of diacylimines even in reactions between amides and acyl halides is proved by the example of the acylation of benzamide by chloroacetyl chloride (2) which gives a good yield of chloroacetyl benzamide. If this reaction would proceed by nitrile formation one would expect the usual exchange of nitrile and carboxylic acid groups to give benzoic acid and chloroacetonitrile which is not the case. It must be stressed, however, that the possibility of this reaction can never be excluded a priori. One may add that some modification of the Gautier reaction might be involved in the Rekshit synthesis of diacylimines (28, 29) on the evidence of the decomposition of potassium amides to nitriles on heating (161).

Treatment of nitrile with hydrogen chloride or bromide leads at first to addition compounds which have been formulated as nitrilium halides, $(RCNH)Cl$ and $(RCNH)Cl.HCl$, by Hentzsch (162,163).

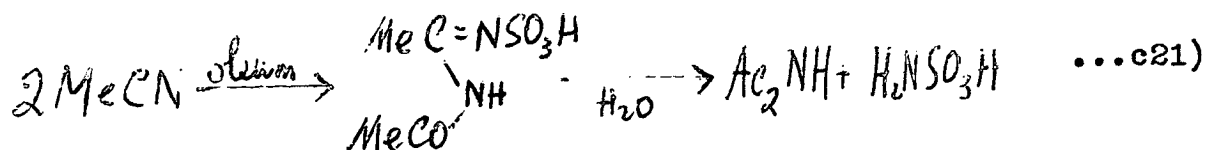
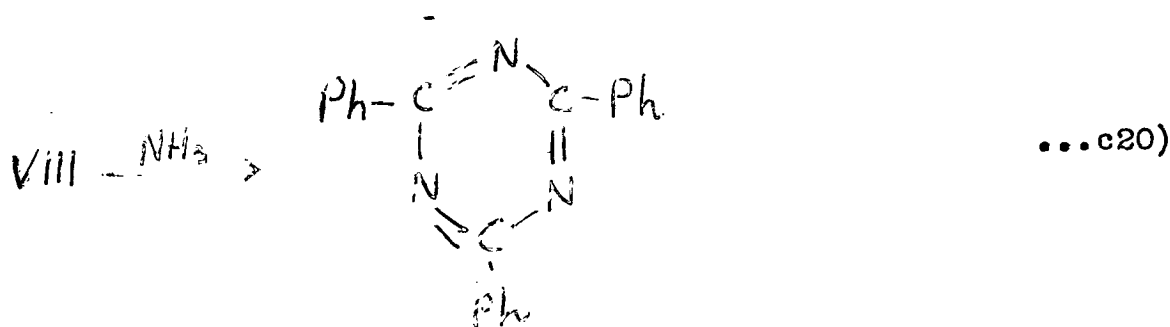
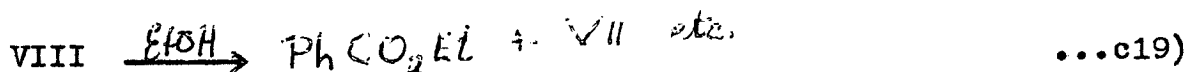
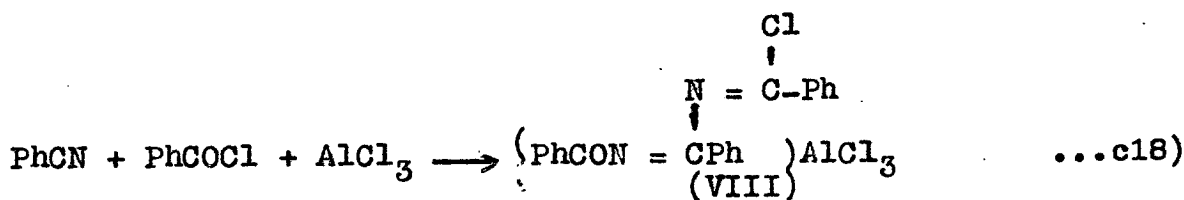
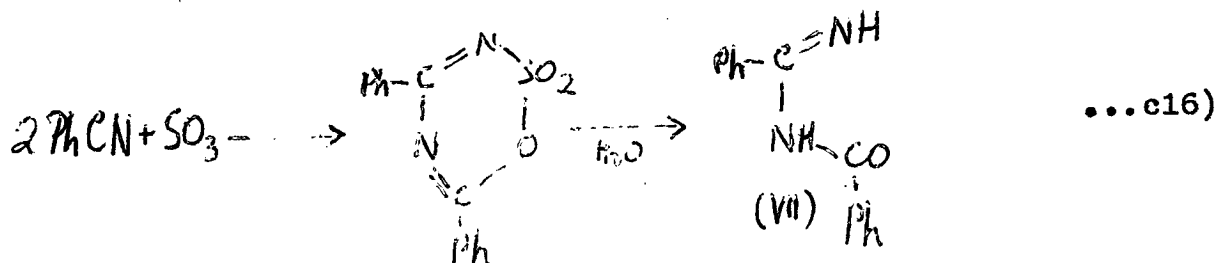
On the evidence of Tröger and Luning (164) these addition compounds decompose on heating and controlled hydrolysis to symmetrical diacylimines (cf. 62):



Similar results were obtained by Engler who reacted acetonitrile with bromine (165, 166). This resulted first in bromination and the formation of hydrogen bromide. The latter brought about reactions similar to those shown in c13-15). The use of acetic acid instead of water on chloroacetimino chloride gives the symmetrical dichlorodiacetimide (167). The formation of a diacylimine from the Houben-Hoesch reaction between phenetole and bromoacetonitrile in the presence of Zinc chloride (168) would be a further confirmation of the Tröger-Luning theory. Unfortunately the product claimed by Houben and Fischer as symmetrical dibromodiacetimide has a much higher melting point than Engler's compound which is supposed to be the same. Work by Francis (169) appears to bear out the correctness of Houben and Fischer's melting point. As regards Engler's product, the author's notes on the melting point of n-butyryl benzamide might apply.

The mechanism of the condensation of nitriles to diacylimines in the presence of strong acids has been best investigated in the case of dibenzimide. After the early work of Hofmann and Buckton,

Engelhardt and Gumpert (170-172) on the action of oleum on phenyl cyanide, Barth and Senhofer (173) worked out a preparation for dibenzimide based on this method. The reaction and its extensions was investigated in detail by Eitner and Krafitt (61, 174-177) whose main results are illustrated in the following reactions:



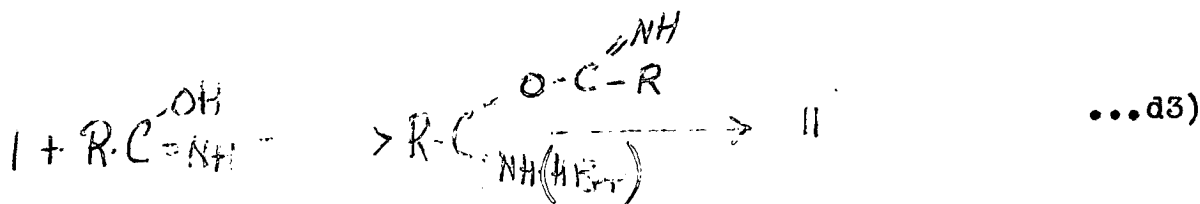
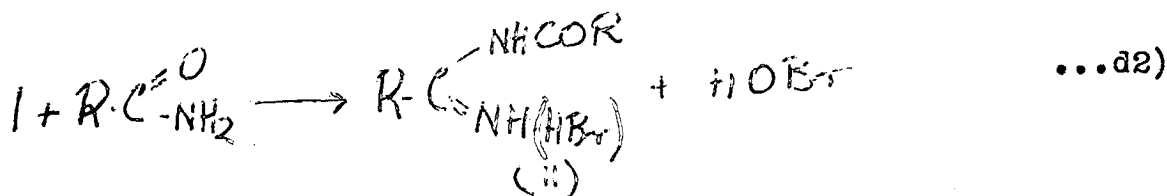
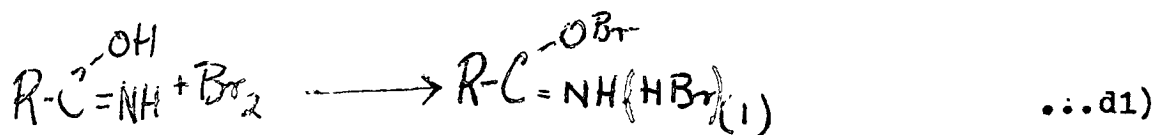
The reaction between propionitrile and propionyl chloride in the presence of aluminium chloride has been studied by Otto and Tröger (178). Although this reaction gives poor yields of dipropionimide owing to side reactions, there is little doubt that diacylimine formation in this case follows the mechanism established by Eitner and Krafft. From the author's experience, modifications of the Gautier reaction are seldom justified for preparative purposes. The reaction of nitriles and acids under pressure is efficient in the aliphatic series but with one or two aromatic reactants side reactions occur and yields are considerably reduced in the course of purifications. This inconvenience is still greater in the case of reactions between nitriles and acyl halides.

d) Other methods of preparation.

Formyl amides may be prepared by condensing amides with formaldehyde under alkaline conditions and oxidizing the resulting N-methylolamides with dilute chromic acid, following the procedure of Einhorn (179,180). The author (with P.L.T.) has repeated some of the experiments of Einhorn and his collaborators. The condensation with formaldehyde usually affords good yields but very great losses occur on oxidizing with dilute chromic acid. The method was found convenient for the preparation of N-formyl benzamide. For the preparation of aliphatic formyl amides methods quoted in part b) of this chapter are preferable.

Diacylimines may occur amongst the products of the Hofmann degradation of amides (181). Unlike the formation of urea derivatives (33) in this reaction, the formation of diacylimines is difficult to explain. In the author's opinion the most likely

explanation is the formation of O-bromides which may be regarded as mixed anhydrides of organic acid and hypobromous acid. This anhydride could then acylate an unchanged molecule of amide:



This theory is in accordance with the fact that diacylimine formation during the Hoffmann degradation is more pronounced in the case of higher amides the enhanced imidol character of which has been discussed before and with the - possibly strained - analogy of the behaviour of benzoyl nitrate which benzoylates amino groups and forms nitrates with alcohols (93). The reactions could be formulated with N-bromoamides although with less plausibility (34).

Following the work of Ruff and Giesel (182), Francis (169) prepared diacylimines from aliphatic acids and sulphur nitride. This reaction is not useful for preparative purposes since the yields are small and amide formation appears to be greater than

the formation of diacylimines. The conversion of ethyl benzoate into dibenzimide by magnesium amide iodide has been described by Odda and Caldero (183). The latter two reactions may be classified formally as acylations but little is known about their mechanism.

4) Physical properties and structure.

a) The melting points of some aliphatic diacylimines and benzamide derivatives are listed in Table 1. Absence of references points to the author's observations recorded in this Thesis.

TABLE 1.

| <u>Diacylimines</u> | <u>Melting Point</u> | <u>Reference</u> |
|-----------------------------------|----------------------|------------------|
| Formyl acetamide | 70° | (129) |
| Formyl chloroacetamide | 89-90° | (179) |
| Formyl propionamide | 65° | (180) |
| Diacetimide | 79-80.5° | |
| Acetyl propionamide | 86° | (2, 146) |
| Acetyl n-butyramide | 119° | |
| Acetyl isobutyramide | 177-178° | (143) |
| Dipropionimide | 154° | (2) |
| Propionyl n-butyramide | 109° | (115) |
| Propionyl isobutyramide | 140° | (115) |
| Propionyl isovaleramide | 68° | (115) |
| n-Dibutyrimide | 111° | |
| n-Butyryl isobutyramide | 103° | (115) |
| Di-isobutyrimide | 174° | (48, 73) |
| Isobutyryl n-valeramide | 84° | (115) |
| Isobutyryl isovaleramide | 94° | (115) |
| Divalerimide | 100° | (114) |
| Di-isovalerimide | 94° | (114) |
| n-Dicaprionimide | 92.5° | (150) |
| Chloroacetyl acetamide | 106° | (2) |
| symm. Dichlorodiacetimide | 195° | (2) |
| Chloroacetyl dichloroacetamide | 98° | (147) |
| Chloroacetyl trichloroacetamide | 80° | (147) |
| Chloroacetyl propionamide | 151° | (2) |
| Chloroacetyl α-chloropropionamide | 108° | (151) |
| Chloroacetyl bromoacetamide | 180° (dec.) | (147) |
| symm. Dibromodiacetimide | (98°) 195° dec. | (165-167) |
| Hexachlorodiacetimide | 81° | (149) |
| Formyl benzamide | 120-121° | |
| Acetyl benzamide | 117° | (2) |
| Propionyl benzamide | 98° | (2, 112) |
| n-Butyryl benzamide | (68-90°) 104-105° | |
| Isobutyryl benzamide | 121° | |
| Isovaleryl benzamide | 89° | (110) |
| Diethylacetyl benzamide | 138-139° | (109) |
| Chloroacetyl benzamide | 157° | (2) |

These few figures show that the melting points of diacylimines do not follow any simple rule in relation to their constitution. It would appear that acylation by a lower acyl rest depresses the melting point of primary amides although propionamide and iso-

butyramide depart from this tentative rule which is hardly significant on the limited evidence.

Few boiling points have been recorded for diacylimines most of which cannot be distilled, except in very good vacuum, without decomposition. Some of these data are subject to doubt on the author's experience and do not warrant further discussion at present.

N-alkylation of diacylimines decreases the melting point and the same applies to N-phenyl diacylimines. N-1-naphthyl diacetimide melts higher than diacetimide but the corresponding 2-naphthyl compound melts lower.

b) Molecular weight determinations on diacylimines lead to the same difficulties and general considerations as similar work on amides (184-191). Low melting diacylimines are monomeric with the exception of N-methyl diacetimide which is dimeric by cryoscopy in camphor. The water soluble diacylimines are monomeric by cryoscopy in water. Students at the University of Tasmania have investigated the molecular weights of a few diacylimines at the author's suggestion. Diacetimide in water is monomeric (R.J.Ford and H.A.Hudson). In benzene the molecular weight increases with concentration as shown in Table 2. (J.L.Davies and M.R.Atkinson).

TABLE 2.

| <u>Concentration (mols/litre)</u> | <u>Molecular weight of Ac_2NH</u> |
|-----------------------------------|--|
| 0 (extrapolated) | 115 |
| 0.127 | 155 |
| 0.193 | 175 |
| 0.246 | 188 |
| 0.500 | 212 |

Dibenzimide (W.D.Jackson) and diacetanilide (G.C.Bratt and R.S.Yos) in benzene are slightly associated. The observed molecular weights

are about 8% higher than the theoretical values with an expected error of $\pm 5\%$. This discrepancy is small and the slight observed variations with concentration may not be regarded as significant. Further data on the molecular weights of diacylimines are being collected by P. Dunn whose results will be included in his M. Sc. Thesis.

c) The quantitative determination of solubilities of diacylimines has been carried out in a few cases only (2). The diacylimines are less soluble in water and more soluble in solvents with low dielectric constants than comparable amides. This indicates less ionization (in the broadest sense) and a reduced tendency for hydrogen bond formation in diacylimines in comparison with amides. This is in accordance with the few observations on the monomeric character of water insoluble diacylimines and with the fact that the melting points and boiling points of diacylimines are not very different from those of either parent amide.

d) Data on the surface tensions of aqueous solutions of diacetimide and the diacetimide-acetamide-acetic acid system in water have been reported before (4, 23). Other diacylimines are either not sufficiently soluble in water to permit accurate surface tension measurements with the author's equipment or they are too unstable in water (N-methyl and ethyl diacetimide).

e) The heats of combustion of some diacylimines have been determined by Parts (192) some of whose results have been confirmed by the author and J. A. T. Cruickshank, Demonstrator at the Chemistry Department of the University of Tasmania. Parts calculated theoretical values for the heats of combustion of

diacylimines from similar values on amides derived from the work of Roth (193, 194). The author recalculated the expected values by Part's method but using somewhat different data on the heats of combustion of amides (195). The results are shown in Table 3.

TABLE 3.

| <u>Compound</u> | <u>Heat of combustion cal/mol</u> | | | |
|------------------------|-----------------------------------|-------------|-------------------|------------|
| | <u>found</u> | | <u>calculated</u> | |
| | <u>Parts</u> | <u>JATC</u> | <u>Parts</u> | <u>JBP</u> |
| Ac ₂ NH | 518.9 | 505.3 | 495.9 | 495.2 |
| (EtCO) ₂ NH | 809.2 | | 809.1 | 807.6 |
| PhCONHAc | 1065.1 | 1065.6 | 1070.1 | 1064.8 |
| (PhCO) ₂ NH | 1634.8 | | 1644.3 | 1634.4 |
| (PhCO) ₃ N | 2425.5 | | 2427.3 | 2412.3 |

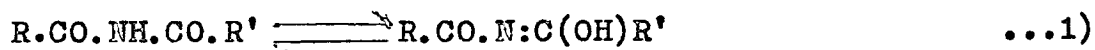
It may be deduced from these data that the resonance energy of dipropionimide exceeds that of diacetimide by about 6 Cal/mol which is in agreement with the difference between the ultraviolet absorption spectra of these compounds. The absolute values of the resonance energies of individual diacylimines cannot be calculated from the available data although the approximate orders may be ascertained by calculating resonance energies for selected tautomeric forms. The results of such calculations are shown for diacetimide in Table 4.

TABLE 4.

| <u>Tautomeric form</u> | <u>Resonance Energy Cal/mol</u> |
|---|---------------------------------|
| 1) CH ₃ -CO-NH-CO-CH ₃ | 1.4 |
| 2) CH ₃ -C(OH):N-CO-CH ₃ | 11.5 |
| 3) CH ₂ :C(OH)-NH-COCH ₃ | 19.1 |
| 4) CH ₂ :C(OH)-N:C(OH)-CH ₃ | 29.2 |
| 5) CH ₃ -C(OAc):NH | 16.6 |
| 6) CH ₃ -C(OAc)NH- | 24.2 |

Forms 5 and 6 refer to "isodiacetimide" which appears to be in tautomeric equilibrium with diacetimide. Form 4 with the highest resonance energy is expected to make a considerable contribution to the tautomeric structure of diacetimide judging from spectrographic data (6), and the modified Perkin reaction which will be considered later.

f) In its classical form the structural problem of amides has been considered from the point of view of amide - imidol tautomerism. The purely chemical approach to this problem has not yielded conclusive results but it has offered actually or potentially useful working hypotheses (25, 26, 60, 197, 198) and was used in Chapter 3 of this Thesis. The most conclusive evidence in favour of the existence of amide-imidol tautomerism is offered by the Raman spectra of amides (199). Contradictory evidence from Raman spectra (200) might be explained with the help of the resonance theory of amides (201). No such data are available for diacylimines but the study of the hydrolysis of diacylimines (35) suggests some form of amide - imidol tautomerism in this class of compounds which, however, need not be as simple as that considered by Titherley



the "enolization" occurring towards the radical R' which is obtained as R'COOH in hydrolytic (35) or R'COOEt in alcoholic (2, 147) experiments.

Ultraviolet spectrography of amides and related iminoethers and N,N-disubstituted amides (162) is frequently quoted in favour of the imidol structure of amides like benzamide and trichloroacetamide. These measurements were semi-quantitative only and

have been criticized by Ramart-Lucas and her collaborators (202-204) whose quantitative measurements still leave the question open. In primary amides "enolization" to the imidol form does not affect the order of conjugation but this effect may be observed in diacylimines since the group CO-NH-CO is not conjugated whereas the group CO-N:C(OH) has a conjugation of the first order. This effect was observed by Polya and Spotswood (6). Later work with P. Dunn confirmed the effect in the case of other diacylimines but since the recently investigated diacylimine exhibit maxima of poor contrast only, it has been decided to defer the publication of these results until they can be checked with a more sensitive instrument which will not be available until 1951. From the spectrographic and chemical evidence it appears likely that the conjugated form of diacylimines is of the form $\text{R.C(OH):N-C(OH):CH}_2\text{R}'$ if at least one of the acyl groups permits enolization. This view is supported by considerations arising out of the modified Perkin reaction between aldehydes and diacylimines (see next chapter).

Such enolized forms have high resonance energies, as mentioned before. It is possible that a similar enolization should be considered to account for the peculiarities of some primary amides. In the author's opinion the following reactions may be governed by enolization effects:

- i) the greater ease of nitrile formation from aromatic than from aliphatic amides;
- ii) the failure of the Gautier reaction between phenyl cyanide and phenyl acetic acid;
- iii) the failure of preparing diacylimines from esters and sodium amides with reactants containing the CH_2CO grouping.

g) In view of the interesting infra-red work carried out by Richards and Thompson (205) and Richards (206) on the structure of amides, similar work would have been desirable in connection with discylimines. Lack of equipment and refusal of the Industrial Chemistry Division of the C.S.I.R.O. to carry out the necessary measurements before 1952 prevent the author from formulating his views on the structure of discylimines in a more definite form.

5) Chemical reactions.

Most of the work reported in this chapter has been carried out in the form of small scale orienting experiments. Work is in progress on most of the reactions recorded in this chapter which is intended to show the potentialities of diacylimines from a synthetic point of view rather than to suggest that the chemical reactions of diacylimines are well known to-day.

a) Alcoholic and hydrolytic experiments have been published before (2, 23; cf. 104) which complement Titherley's work on the alkaline hydrolysis of diacylimines (35). The results are in agreement with the finding of König (147) that on the alcoholysis of unsymmetrical diacylimines the stronger acid rest joins the alkoxy-group and the weaker acid rest retains the nitrogen. Work with P.L.T., M. Atkinson and Dr. A. Komzak indicates that this rule holds good in the acylation of hydrazines by unsymmetrical diacylimines although the reaction does not stop at that stage.

b) Unsymmetrical diacylimines undergo some rearrangement to a mixture of symmetrical diacylimines on heating but this reaction is very slight compared with an identical rearrangement of mixed anhydrides (2).

c) Diacetimide in saturated aqueous solution was shaken with 2-3 parts by weight of mercuric oxide for 2-4 hours. After filtering and extracting the insoluble portions with absolute alcohol in a Soxhlet apparatus tetraacetyl hydrazine, m.p. 85° , was isolated in yields of 0.6-3.1% from various experiments.

M/100 amounts of diacetimide were refluxed for 2 hours with 1 g. potassium permanganate and 100 cc. glacial acetic acid. The acetic acid was removed by distillation (the last 10-20 cc.

in vacuum) and the residue was extracted with ether to remove unreacted diacylimides. The dry residue was ground and extracted with 10 lots of ice cold ethanol (15 cc. each). The time of contact for each extraction was limited to 1 minute. The combined alcoholic extracts were dried in vacuum and extracted with 50 cc. ethanol. The colourless extract was dried in vacuum and recrystallized from acetone. Succinimide, m.p. 126° , was obtained in yield of 24-38%. Experimental difficulties in analogous reactions with other diacylimines have not been overcome so far.

Aniline or p-nitroaniline were diazotized in M/10 amounts. The filtered solutions of diazonium halides were added to the calculated amount of diacetimide dissolved in acetone in the presence of sufficient solid sodium acetate to keep the pH at 6 - 6.5. A vigorous reaction occurred; the mixture turned dark and nitrogen was evolved. Phenylhydrazine or p-nitrophenylhydrazine were isolated from the reaction mixture in addition to small quantities of succinimide. The yields were poor (7-12% of the hydrazines and 1-2% of succinimide) and most of the product consisted of a dark resin which could not be recrystallized or distilled.

d) Reduction of diacylimines by zinc with acid or alkali and under Clemmensen conditions failed. Reduction by the Bouveault-Blanc method (38, 39) gave small amounts of primary amines in addition to larger amounts of esters. The latter were noted qualitatively only. The amines were isolated by distilling the volatile portions from acidified solutions and then distilling the amine after the addition of excess alkali

into standard sulphuric acid. On determining the amine nitrogen content by titration the amines were converted into the benzoyl derivatives and weighed. Since qualitative tests excluded the presence of secondary amines, these determinations permitted the calculation of yields when a mixture of amines was possible. The yields of amines from various diacylimines are shown in Table 1.

TABLE 1.

| <u>Diacylimines</u> | <u>Amines</u> | <u>Yields</u> |
|------------------------|-----------------------------------|---------------|
| Ac ₂ NH | EtNH ₂ | 5% |
| EtCONHAc | EtNH ₂ | 3% |
| | PrNH ₂ | 4% |
| (EtCO) ₂ NH | PrNH ₂ | 6% |
| PhCONHAc | EtNH ₂ | 1% |
| | PhCH ₂ NH ₂ | 0.3% |
| (PhCO) ₂ NH | PhCH ₂ NH ₂ | trace onl |

In spite of the unavoidable inaccuracy of the analytical process these figures indicate the nature of the reaction.

e) Diacetimide does not react with liquid bromine in the cold. On heating hydrogen bromide is evolved and a mixture of brominated acids and nitriles is obtained. Diacetimide and bromine in pyridine solution do not react in the cold. If the solution is refluxed for 2-4 hours the products are similar to those obtained in the preceding experiment. If a drop of aqueous alkali is added to a solution of diacetimide and bromine in pyridine a vigorous reaction sets in after a few minutes and may proceed with explosive violence. Careful fractionation of such reaction mixtures established the presence of acetic acid, bromoacetic acid, tribromoacetic acid, acetonitrile, bromo-

acetonitrile and tribromoacetonitrile (not all of which have been identified in the same experiment) in addition to brominated pyridine bases amounting 3.6-6.4% of the pyridine which was used in a 4M excess over equimolar amounts of diacetimide and bromine.

If concentrated solutions of equimolar amounts of diacetimide and bromine in ether are mixed and allowed to stand over night a heavy dark layer separates. On keeping the oil over solid potassium hydroxide in a desiccator for a few days the oil solidifies to a yellow, resinous mass. On further standing fine white needles grow upwards. Small amounts of these needles were collected from several experiments. They had melting points ranging from 130.5° to 136° in various experiments. The bromine content was determined in one case only since most of the other crops were available in very small amounts only.

Bromine found : 45.6%, calculated for $C_4H_6O_2NBr$: 44.44%.

The substance gave a positive hydroxamic acid reaction and contained little or no "positive bromine". Provisionally the substance is regarded as $BrCH_2CONHCOCH_3$ which has not been described before. The resinous residue gave a positive hydroxamic test and contained "positive bromine". It could be recrystallized from chloroform in poor yields. The resulting white crystals (with a faint yellow tinge) melted at $113-115^{\circ}$ with decomposition. Bromine found : 45.2%, calculated for $C_4H_6O_2NBr$: 44.44%. All the bromine is contained as positive bromine (45.1% by iodometric titration). On treatment with dilute sodium bicarbonate in the cold the substance decomposes and yields diacetimide. In view of these data the substance

is regarded as α -bromodiaceitimide.

For a more convenient preparation of N-bromodiacetimide one cools a saturated solution of sodium bicarbonate (100 cc.) in an ice-salt bath and adds diacetimide (10 g.) in water (50 cc.) followed by bromine (16 g.) with good stirring. Carbon dioxide is evolved and the mixture sets to a thick paste in a few minutes. Stirring is continued for another 20-30 minutes until the colour is a uniform light yellow. The mixture is filtered by suction and washed free from traces of bromine and unreacted diacetimide with small amounts of ether. N-bromodiacetimide is slightly soluble in ether but loss of the desired material in this operation is unavoidable if material free from diacetimide and bromine is wanted. The washed residue is nearly white when fresh but on standing some decomposition occurs with the liberation of bromine. It is advisable therefore to proceed with the purification without delay and to use the material while fresh. The washed residue is contaminated by inorganic matter (mainly sodium bromide). It can be extracted with perfectly dry acetone. On removing the acetone under vacuum an oily residue is left which solidifies rapidly in a vacuum desiccator charged with solid potassium or sodium hydroxide. The melting point is not sharp (owing to decomposition and possibly traces of acetone: $112-115^{\circ}$) but it is not depressed when mixed to the compound prepared by the preceding method. Yields of purified N-bromodiacetimide are of the order of 16-20% by this method.

On prolonged keeping the substance turns dark red but this is not due to free bromine. In fact the "positive bromine" content of old, resinous sample is very low. The hydroxamic

test becomes negative after prolonged standing. Recrystallization of old preparations from chloroform may recover a small fraction of the compound. The use of N-bromodiacetimide in Wohl-Ziegler reactions is being investigated. It will be sufficient at present to report that in a few preliminary experiments with N.K. Vallance toluene and N-bromodiacetimide were found to give both bromotoluenes and benzyl bromide the former predominating. No precautions have been taken to avoid peroxide effects. In reactions with pyridine mixtures of bromopyridines are obtained after short heating. These mixtures have not been fractionated at the time of writing.

f) If a solution of ethylmagnesium bromide in ether is added to an equimolar amount of diacetimide in ether with good stirring the N-magnesium bromide derivative of diacetimide precipitates in quantitative yield accompanied by evolution of gas (with J.Davies). Found N : 6.83%, calculated : 6.85%; found Br : 39.17%, calc. : 39.15%; found Mg : 11.93%, calcd. : 11.90%. After 4 months' standing the substance contained 38.96% bromine (N.K.Vallance). Reaction with isobutyl bromide gave an oil with a pleasant camphor odour which did not analyse correctly for N-isobutyl diacetimide. The purification of the oil was not attempted but it was hydrolysed by dilute hydrochloric acid. The hydrolysed product was worked up by J.L.Davies who obtained from it isobutyl amine in a yield of 22%. Methyl iodide in a similar reaction afforded small amounts of a neutral liquid boiling at 100-110° which could not have been N-methyl diacetimide. Acetyl chloride gave an oil with anise odour. The latter two products have been obtained in poor yields and in obviously impure forms and are unidentified as yet.

Myristyl, cetyl and stearyl bromide were reacted with the N-magnesium bromide derivative of diacetimide. In the absence of solvents much decomposition occurs and the reaction is too slow when ether or benzene are used as solvents. The N-magnesium bromide derivative of diacetimide reacts much more slowly than Grignard compounds even with reactants like acetyl chloride.

g) A note on the fluorimetric assay of some halogenated diacylimines has appeared earlier (5). The copy of a paper on the colorimetric assay of diacylimines, which has been accepted by the Analytical Journal, is attached to this Thesis.

h) It was shown in an earlier publication (3) and two papers, which have been sent to *Receuil des travaux chimiques des Pays-Bas* a little while ago and copies of which are attached to this Thesis, that diacylimines may replace anhydrides in the Perkin reaction. The use of catalysts is necessary to obtain optimum yields but the condensation occurs also in the absence of catalysts. This is in agreement with the previously expressed views (6) on the appreciable contribution of enolized forms to the tautomeric structure of diacylimines with enolizable acyl rests. In the modified Perkin reaction bis-amides of the aldehydic reactant occur as by-products. The occurrence of this side-reaction is not difficult to explain but no explanation has been found for the empirical fact that the formation of bis-amides is of the same order as that of the unsaturated amides if potassium acetate is used as catalyst while the yield of bis-amides is negligible with sodium acetate as catalyst.

It was not intended to report in this Thesis further investigations in this field which are far from complete but a recent paper by Dippy and Evans (249) may necessitate a few

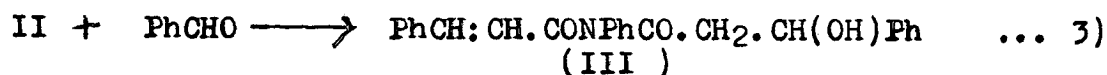
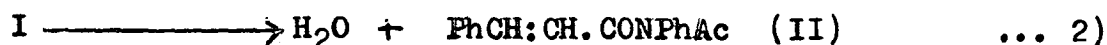
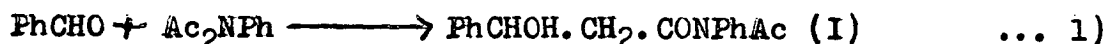
comments which involve reference to work in progress (with T.M.S.)

i) In view of the possibility of the uncatalyzed condensation of diacylimines with benzaldehyde and the similar mechanism of the original and modified Perkin reactions the catalytic effect of sodium or potassium salts of weak acids may not be regarded as the main drive of the condensation unless it is considered in conjunction with the enolizability of the non-aldehydic reactant.

ii) Dippy and Evans have obtained cinnamic acid from benzaldehyde and acetamide in the presence of sodium acetate in a yield of less than 1%. The author with P.L.T. did not obtain any cinnamic acid in a similar experiment. In view of the small yield of cinnamic acid this discrepancy is not serious. It is surprising however, that Dippy and Evans did not note the formation of benzylidene bis-acetamide and cinnamamide. N,N-dimethyl acetamide for instance gives N,N-dimethyl cinnamamide when condensed with benzaldehyde in the presence of sodium or potassium acetate. In this case the formation of the bis-amide is excluded, of course.

iii) Dippy and Evans have obtained cinnamic acid in a yield of 18% from the condensation of benzaldehyde and diacetanilide in the presence of sodium acetate. The author with T.M.S. noted the same before seeing the paper of Dippy and Evans but the formation of N-phenyl cinnamamide was observed at the same time. Although the optimum conditions for this reaction have not been established so far, the yield of N-phenyl cinnamamide afforded by this reaction is at least of the order of 35-40%. The lower yield of cinnamic acid is in agreement with the author's view that the first stage of the reaction gives cinnamoyl acetanilide which

may react with more benzaldehyde to the unsymmetrical diacylimine III which loses cinnamic acid in the usual manner :



These reactions refer to the experiments of Dippy and Evans . Under the author's conditions the dominant reaction appears to be



On the arguments of Polya and Spotswood (Amides VII ., attached to this Thesis) elimination of acid from the primary condensation product is more likely than the elimination of water .At the same time the reaction between benzaldehyde and diacetanilide suggests that elimination of water may occur to some extent .

iv) It must be admitted that the retention of the amide group in modified Perkin condensations involving reactants with the $-\text{CH}_2 \cdot \text{CONH}-$ grouping can not be postulated as a general rule .

Thus the author with T.L.Lewis obtained low yields of coumarin from the condensation of salicylaldehyde and diacetimide in the presence of sodium acetate.The main product was a red , brittle resin which was resistant to strong acids and alkali and was insoluble in all the usual organic solvents.Although the constitution of this product is unknown (apart from the fact that it contains nitrogen),the formation of coumarin proves the loss of amido nitrogen in the reaction .

6) Acetyl phosphamide.

The preparation of N-benzoyl phosphamide has been described by Titherley and Worrall⁽²²⁰⁾. Other N-acyl phosphamides do not appear to have been reported in the literature although acetyl phosphamid should be of some interest owing to its formal similarity with acetyl phosphate (8, 9). It was intended at first to prepare phosphamide and to acetylate it with acetic anhydride or acetyl chloride, possibly in the presence of catalysts. These experiments have been abandoned for the time being after noting a more convenient way of preparing the disilver salt of acetyl phosphamide. The work reported in this chapter has been carried out with G.C. Bratt who performed the synthetic operations and analyses under the author's direction. The latter has carried out the other tests reported in this chapter and in a paper by P.L.T. and the author which will be published shortly in Analytical Chemistry and a copy of which is attached to this Thesis. Studies on the biochemical properties of acetyl phosphamide are being carried out by Miss E. Ashbolt and the author and will be reported briefly only.

Diphenyl monophosphamide was prepared by the method of Audrieth and Toy (221) in a yield of 33%. It had a melting point of $142-3^{\circ}$ instead of $145-6^{\circ}$ (221) or 148° as claimed by Stokes (222). It contained some triphenyl phosphate as the extraction of the latter with cold carbon tetrachloride does not appear to be very efficient. Diphenyl monophosphamide was converted into phosphamide by Stokes' method (222, 223). The substance was refluxed with the calculated amount of 10% potassium hydroxide solution for 15 minutes, precipitated with a slight excess of

saturated lead nitrate solution and the precipitate was decomposed in an ice-cold suspension with hydrogen sulphide. The treatment with hydrogen sulphide was repeated after filtration to make sure of complete decomposition. On standing in the refrigerator over night phosphamide separated as a fine powder which decomposed without melting at about 230° . On prolonged chilling crystalline material could be obtained. The yields from several experiments varied around 2% only.

In another series of experiments attempts were made to convert ammonium fluorophosphate (224) to diammonium phosphamide by liquid ammonia. Separation through the thallous salt failed and separation through the silver salt afforded disilver phosphate only although the latter might have been contaminated with a trace of the desired compound.

Finely ground diammonium phosphate (10 g.) was mixed with 85% phosphoric acid (8.75 g.) and refluxed with acetic anhydride (25.5 g.) and acetyl chloride (1 g.) with intermittent shaking for 1 hour. The volatile portions were removed by distillation, the residue was dissolved in water and neutralized to phenol red with 33% sodium hydroxide solution. Addition of 25 cc. of 5% silver nitrate solution to the hot solution gave an immediate white precipitate which was discarded. The cooled solution was treated with 25% silver nitrate solution (50 cc.) in small portions. A precipitate was formed on each addition but dissolved rapidly. On adding more silver nitrate (325 cc.) a permanent white precipitate was formed in a yield of 37 g. (61%).

Found Ag : 60.6%, P : 8.65%, $\text{CH}_3\text{CO-}$ 12.0%; calculated for disilver phosphamide Ag : 61.0%, P : 8.85%, $\text{CH}_3\text{CO-}$: 12.21%.

The substance contains nitrogen and does not give the Lipmann-Tuttle test for acetyl phosphate (7). On exposure to atmospheric moisture acetamide is liberated. With a slight excess of 10% sodium chloride solution silver chloride precipitates and the filtrate presumably contains the sodium salt of acetyl phosphamide. The preparation of pure acetyl phosphamide was not achieved so far. The decomposition of the silver salt with hydrogen sulphide was incomplete and decomposition with thioacetamide was unsuccessful. However, the original disilver salt may be reprecipitated from aqueous solutions of sodium acetyl phosphamide without loss while recoveries from aqueous solutions of acetyl phosphamide freed from silver with hydrogen sulphide or thioacetamide are poor (14-29% of the original disilver salt). Thionyl chloride was used instead of acetyl chloride in another experiment which afforded less stable, discoloured material.

Although fresh solutions of sodium acetyl phosphamide do not give the Lipmann-Tuttle test, a faint positive reaction is obtained if the solutions are allowed to stand for 3-4 hours at room temperature. On longer standing the test becomes negative and precipitation with silver nitrate gives yellow silver phosphate in addition to the white precipitate of disilver acetyl phosphamide.

Sodium acetyl phosphamide in a concentration of 0.1% inhibits the fermentation of glucose by a pure strain of brewer's yeast (Karlsberg 126 P.T.) although fermentation may start after a delay of a week or more.

An aqueous solution containing 0.5% L-glutamic acid and 0.01% sodium acetyl phosphamide becomes viscous on standing.

The surface tension decreases rapidly to 55-57 dyne/cm at 20° within a few minutes then more slowly to 45-49 dyne/cm within 3-4 days. These preliminary observations by the author are being followed up in collaboration with A.E. Parkes at present.

III. The Brunner Reaction.

At the time of initiating studies on diacylimines in Hobart the only distinctive reaction of these compounds noted in the literature was that with hydrazine derivatives to give substituted 1,2,4-triazoles. This reaction was discovered by Brunner (32, 71) whose school continued the study of this reaction for a short time. Most of that work was concerned with the preparation of new triazoles and with the substitution reactions of some triazoles. A partial elucidation of the reaction mechanism was based on the observation that the reaction between diacetimide and semicarbazide gives the amide of 2,5-dimethyl-1,2,4-triazole-1-carboxylic acid as an intermediate which on heating in aqueous solution undergoes hydrolysis and decarboxylation to afford 2,5-dimethyl-1,2,4-triazole as the final product (207). This observation is of little value when considering the reaction of diacylimines with $RNHNH_2$ ($R = H$, alkyl or aryl) which cannot occur with a loss or modification of R . Conditions of the reaction were studied by Brunner and his collaborators (208-210); these were substantially confirmed and somewhat extended in the author's studies. It appears that the Brunner reaction in its original form has not been used by other investigators. This is probably due to the fact that while the preparation of the necessary diacylimines is simple on paper the best yields can be obtained only by workers with considerable experience, and more readily available starting materials may be preferable if synthetic studies on triazoles do not involve the investigators' interest in the chemistry of amides. Recent work by Kaiser on the preparation of 3-amino and 3-ureido 1,2,4-triazoles from

acyl dicyanodiamides and hydrazine (211, 212, cf. 213) has analogies with the Brunner reaction although the reaction mechanism is obviously different.

The interesting biological properties of some 1,2,4-triazoles were noted early during the author's studies. This has led to repeated preparations of a few selected 1,2,4-triazoles for biological tests, some of them by methods other than the Brunner synthesis. As a consequence of this and of the discontinuity of work owing to changes of staff the chemical work on the Brunner reaction has not progressed very far although most of the work in progress in the author's laboratory is concerned with this reaction at present.

Preliminary experiments in this field have been carried out by the author. While on the teaching staff of this Department, P.L. Tardrew carried out much preliminary work on this subject. His work included the preparation of a number of intermediates for the synthesis of 1,2,4-triazoles by the Brunner reaction and other methods, studies on the effect of basic reagents on the course of the reaction and the compilation of an extensive bibliography on 1,2,4-triazoles. All this work forms part of a M. Sc. Thesis by P.L.T. The author worked out methods of isolation and purification which will be given below. Dr.A. Komzak and the author studies the Brunner reaction between diacetimide and phenylhydrazine under varied conditions. M.R. Atkinson with the author carried out a few experiments on the Brunner reaction of unsymmetrical diacylimines. Biochemical work involving 1,2,4-triazoles will appear in Section IV.

As described by Brunner (71), the reaction between

phenylhydrazine and diacetimide affords 1-phenyl-3, 5-dimethyl-1,2,4-triazole (PDMT). Brunner pointed out that this reaction established a formal analogy between diacetimide and β -diketone substances. The same analogy applies to other diacylimines, although less conclusively to aromatic diacylimines which react sluggishly (208). In the author's opinion, however, the latter observation by Wolchowe is not strictly accurate as it may be due more to the insolubility of aromatic diacylimines in water than to fundamental structural differences.

In our experiments equimolar amounts of phenylhydrazine and diacetimide were used, as the author's preliminary experiments have shown that the use of 3 mols of the latter (214) does not appreciably improve yields but renders theoretical consideration more difficult. The reactants are dissolved in a suitable solvent and refluxed for a few hours. Solvents other than water (alcohol, pyridine, benzene) are removed by steam distillation. Although PDMT is volatile in steam the losses in such a process are slight. It has been found that there is a time-lag of a few minutes between the removal of the solvent and the beginning of the steam distillation of PDMT.

In small scale experiments, particularly when products other than PDMT must be identified, it is best to precipitate PDMT from its aqueous solution with a hot 5% solution of mercuric chloride. PDMT.HgCl_2 is obtained on standing as a light brown microcrystalline powder which may be recrystallized from hot water to give white needles. The composition claimed by Brunner has been confirmed by analysis (L.N.R. Atkinson). Hg found 43.9%, calculated 44.1%; Cl found 16.3%, calculated 16.0%.

The crude mercuric chloride complex melts between 175-190°. Brunner recorded the melting point 187-8°. This has been confirmed in our experiments (186-188°). Samples melting higher were found to contain traces of infusible matter (probably mercuric chloride). The mercuric chloride complex is digested with 2.5-4 parts of cold concentrated hydrochloric acid which leave a little dark tarry residue undissolved. After filtering through sintered glass the solution is made alkaline and extracted with ether. It was found best to neutralize with concentrated aqueous ammonia to a faintly acidic reaction and complete the alkalization with sodium carbonate. The resulting precipitate is coarse, easily filtered and does not retain much absorbed PDMT. If ammonia alone is used for neutralization PDMT precipitates in oily droplets around pH 6 - 7 together with basic mercury compounds and absorption of the former on the fine precipitate of the latter leads to losses.

For preparative purposes it is best to make the aqueous residue from the steam distillation alkaline and extract with ether. The contents of the ether solution are then fractionated in vacuum. The main fraction is collected at 150-200°/20 mm and redistilled at 163-165°/25 mm or 150-154°/20 mm. The redistilled material is of good quality. It may be recrystallized from petroleum ether (with seeding!). The highest melting point observed with pure PDMT was 45-7°. Less carefully purified preparations melt at 43° as recorded in the literature.

In comparing the effectiveness of various conditions the yield of PDMT may be considered in two ways. The yield as mercuric chloride complex is a measure of the true efficiency of the reaction. Actual yields of pure are lower, of course.

It appears from a number of experiments that the yield of pure base is 75% of that of the mercuric chloride complex. The major organic byproducts of the reaction are acetamide and 1-phenyl-2-acetylhydrazine. The former has been quantitatively determined in an experiment with pyridine as solvent. The latter accompanies PDMT and may be separated from it either by the mercuric chloride method or fractional distillation. It is also very much less soluble in ether or petroleum ether than PDMT which may be used for purposes of purification. The following conditions were studied.

a) Reactants.

Diacetamide reacts with phenylhydrazine hydrochloride to PDMT but no PDMT is obtained if the free hydrazine base is used. Thus in a 0.4M experiment in pyridine no PDMT was formed but 0.29 mols of acetamide and 0.34 mols of 1-phenyl-2-acetylhydrazine have been recovered after recrystallization. The corresponding crude products were obtained in 0.41 and 0.35 molar yield respectively. It appears therefore that under these conditions the reaction follows practically quantitatively the equation



1-Phenyl-2-acetylhydrazine was the only identifiable product in a number of other experiments of P.L.T. using alcoholic sodium ethoxide, sodium in benzene or xylene, sodium ethoxide in xylene and sodium acetate (dry) in benzene with diacetamide and phenylhydrazine hydrochloride. The failure of the reaction with alcoholic sodium ethoxide has been confirmed with Dr. A. Komzsek. In a 0.02 M experiment (with Dr. A. Komzsek) using 20 cc. N/1 sodium hydroxide the final pH after 4 hours' refluxing was about 6. This experiment did not afford PDMT ; 1-phenyl-

2-acetylhydrazine and acetamide have been isolated without determining yields. These observations confirm and somewhat extend Brunner's observation (71) that hydrazine salts are necessary for the success of the reaction. Since Kaiser's formally analogous syntheses (211-213) are being carried out in alkaline media it would appear that the mechanism is different in the Brunner and Kaiser syntheses.

b) Solvents.

The best results were obtained in water containing 7.5% acetic acid and an equimolar amount of sodium acetate. Yields in pyridine were somewhat lower and yields decreased further in water alone, in 50% acetic acid and in glacial acetic acid (in this order) but glacial acetic acid in the presence of dry sodium acetate gave a good yield.

c) Time.

Short reaction times of 30-60 minutes give negligible yields. In pyridine the yield after 4 hours' refluxing is 42% and after 8 hours 35% only. In the case of water with acetic acid - sodium acetate buffer the effect of time is less clear. The yields after refluxing for 4, 8 and 16 hours were 39%, 33% and 55% respectively.

These experiments on the preparation of PDMT from equimolar amounts of phenylhydrazine hydrochloride and diacetimide are summarized in Table 1.

Table 1.

| <u>Mols</u> | <u>Solvent</u> | <u>Time</u> <u>Hours</u> | <u>Yield PDMT</u> | | <u>Yield</u> <u>PhNHENHAc</u> |
|-------------|--------------------------|-----------------------------|---------------------------------|---------------------|----------------------------------|
| | | | <u>as PDMT.HgCl₂</u> | <u>as pure base</u> | |
| 0.1 | Pyridine 50 cc. | 3 | 35% | 26% | ... |
| 0.1 | " | 4 | 56% | 42% | ... |
| 0.1 | " | 8 | 47% | 35% | ... |
| 1.0 | Pyridine 500 cc. | 4 | ... | 26% | ... |
| 0.02 | Water 10cc. | 4 | 46% | 35% | 11% |
| 0.02 | AcOH, 10cc. | 4 | 32% | 24% | ... |
| 0.02 | 50% AcOH, 10 cc. | 4 | 41% | 31% | 4% |
| 0.1 | 15cc. AcOH 8 g. AcONa | 16 | 59% | 34% | ... |
| 0.02 | Buffer 20 cc | 4 | 52% | 39% | ... |
| 0.1 | Buffer 160 cc | 8 | 44% | 33% | 4% |
| 1.0 | Buffer 800 cc | 16 | ... | 55% | 13% |

The yields of all these experiments could be improved by pooling the appreciable amounts of discarded material from purifying operations from several experiments. It is estimated that this would increase the quoted yields by a factor of about 1.1 - 1.15.

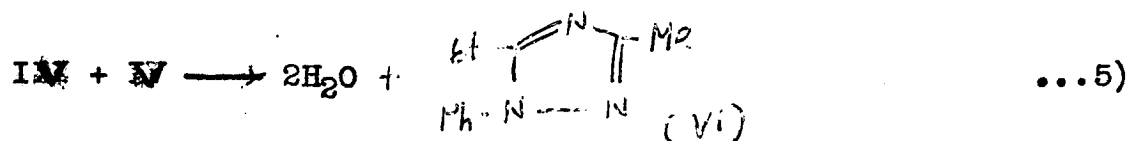
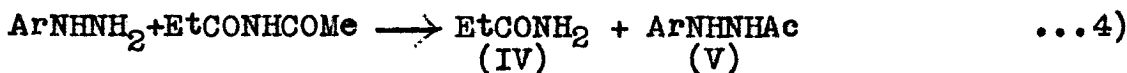
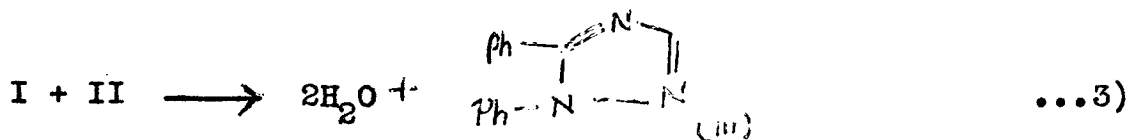
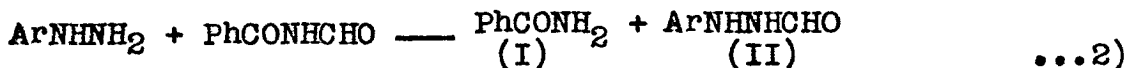
An analogous experiment (with E.R. Atkinson) using p-nitrophenylhydrazine with diacetimide and pyridinium chloride in pyridine afforded yields of around 50% of 1-p-nitrophenyl-2,2-diacetylhydrazine but not triazole. The reaction of formyl benzamide with phenylhydrazine hydrochloride in pyridine (with

M.R. Atkinson) could yield 1,3-diphenyl-1,2,4-triazole and/or 1,5-diphenyl-1,2,4-triazole both of which have been recorded before (215, 216) but only the latter was obtained in a yield of 52%, m.p. 90-91^o picrate m.p. 139^o.

Acetyl propionamide and phenylhydrazine hydrochloride were reacted in buffer solution (with P.L.T.) and in pyridine (with M.R.A.). The product obtained with P.L.T., could be isolated in very small amounts and was found identical with 1-phenyl-3-methyl-5-ethyl-1,2,4-triazole prepared by fusing 1-phenyl-2-acetylhydrazine with propionamide (217). In the experiment with M.R.A. we obtained an oil in a yield of 48% which appeared to be a mixture of both isomers although approximating more to 1-phenyl-3-methyl-5-ethyl-1,2,4-triazole than to its isomer prepared by the fusion of 1-phenyl-2-propionylhydrazine with acetamide. This argument is based on the melting points of the picrates : 3-methyl-5-ethyl compound, m.p. 138^o; 5-methyl-3-ethyl compound, m.p. 132-133^o; product by the Brunner reaction, m.p. 140-142^o. Also the mercuric chloride complexes of the 3-methyl-5-ethyl compound and of the unknown product are formed more readily than that of the 5-methyl-3-ethyl compound.

The formation of 1,5-diphenyl-1,2,4-triazoles and the probable formation of 1-phenyl-3-methyl-5-ethyl-1,2,4-triazole in our experiments suggested that the reaction may be identical with the Pellizzari synthesis of 1,2,4-triazoles from acyl hydrazines and amides. The reaction takes place under near-neutral conditions which, on previously outlined evidence (2 and II.3b of this Thesis), should lead to acylation by the stronger acyl rest of mixed diacylimines with the formation of the amide of

the weaker or aromatic acid thus providing the reactants for a Pellizzari reaction (218).



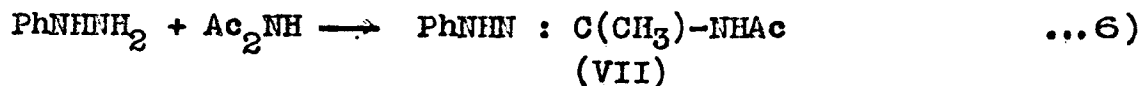
The possibility was considered that the formation of the triazoles III and VI from I and II or IV and V may be inhibited in the presence of basic compounds thus accounting for the failure of the Brunner reaction under basic conditions. In order to test this hypothesis acetamide and 1-phenyl-2-acetylhydrazine were reacted together under the conditions of the Brunner reaction in pyridine in the presence of pyridinium chloride, buffer or water. In all these cases the reactants could be recovered unchanged. The Brunner reaction therefore is distinct from the Pellizzari synthesis of 1,2,4-triazoles.

A further possibility was that the acyl hydrazine, assumed to be formed as the first step of the Brunner reaction, requires a diacylimine instead of a primary amide to form the 1,2,4-triazole. However only the unchanged reactants could be recovered from an attempted reaction between 1-phenyl-2-acetylhydrazine

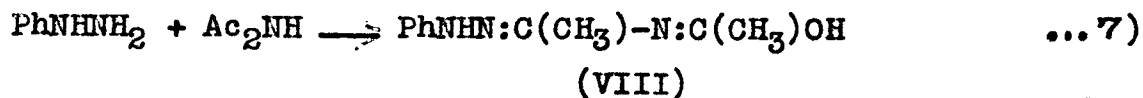
and diacetimide in glacial acetic acid (with P.L.T.).

If the Brunner reaction proceeded by the 1-acylation of arylhydrazines, as considered by P.L.T. in his M. Sc. Thesis, one would expect the enhancement of 1,2,4-triazole formation on substituting p-nitrophenylhydrazine for phenylhydrazine which is not the case. Also the preparation of 1-aryl-1-acylhydrazines by the acylation of arylhydrazines is difficult and no such 1-acylhydrazines could be detected in our experiments.

On the limited available evidence the following mechanism appears most likely. Under acid or base catalyzed conditions the hydrazines are acylated by diacylimines to give the reactants of the Pellizzari reaction which cannot react further at the low temperatures used in the Brunner reaction. In buffered media 1,2,4-triazoles may be formed either in one step or through the "arylhyazones" of diacylimines



or



Either VII or VIII could cyclize to 1,2,4-triazole. Such a reaction is easier to formulate with VIII but the experiment with p-nitrophenylhydrazine points to the likelihood of VII as an intermediate although the exact course of the reaction cannot be formulated with certainty at present. It appears certain, however, that acylation and the normal Brunner reaction are competing processes. Some experiments of Brunner (209, 210)

suggest that the intermediate formation of hydrazidines may play a role. From a quantitative point of view this type of reaction does not appear to be very likely (209) and no hydrazidines have been noted in our experiments. At the same time this point deserves further attention and will be studied in the near future.

IV. Biochemical studies.

The major results of the author's biochemical investigations are contained in two papers copies of which are attached to this Thesis. The former, with P. Dunn, (4) deals with a modification of the melostagnin reaction for cancer based on the use of reagents with low surface activity. Amongst reagents of this kind which have been tested by the author acetamide alone was found to be satisfactory. The latter paper, which has been sent for publication, has been written together with W.D. Jackson who, on the author's suggestion has tested the cytological effects of a few 1,2,4-triazoles and alkylated hydrazines (obtained as by-products in the triazole syntheses). While W.D. Jackson was guided in his work by Professor H.W. Barber (Botany Department, University of Tasmania) and the author, he is solely responsible for the cytological experiments (including the preparation of drawings and microphotographs), much of the purely cytological discussion and the initiation of the use of 1-phenyl-3,5-dimethyl-1,2,4-triazole as an auxiliary reagent in a fast process of counting elongated chromosomes. W.D. Jackson is expected to graduate at the time of submitting this Thesis; further details of his cytological work on 1,2,4-triazoles are being reserved towards a post-graduate Thesis. The paper with W.D. Jackson contains references to preliminary results of the author's investigations with Miss E. Ashbolt and E.A. Parkes and to other biological tests which have been carried out at other Institutes at the author's request. Further notes of this kind have been recorded in a paper by Polya and Spotswood (2). Details of the author's work with E.A. Parkes are contained in the Honours Thesis of the latter. For these reasons this section

is limited to a brief summary of results concerning the extension of earlier work on the melostagmin reaction (4) which has been studied with E.A. Parkos. The figures have been prepared by the latter.

The test is very sensitive to the grade of purity of acetamide. Recrystallization of distilled acetamide from benzene and ethyl acetate (225) is satisfactory if care is taken to remove traces of solvent by heating in vacuum and storing the material in a desiccator over potassium hydroxide and paraffin chips. Precipitation of distilled acetamide from acetone solution with petroleum ether gave material which, by chemical tests alone, appeared to be as pure as the former preparation which, however, was unsatisfactory for the purposes of the melostagmin reaction for the following reasons. It gave variable, slightly but significantly lower, surface tensions in aqueous solution than the former preparation. Melostagmin tests with the precipitated material could not be duplicated, most tests gave negative C values (see 4) for cancer sera and multiple maxima (or points of inflexion suggesting additional maxima) appeared in a number of tests. Similar difficulties were experienced with recrystallized material retaining traces of solvents but such material became normal on the careful removal of residual solvent whereas the reprecipitated material remained abnormal even on prolonged drying. It was noted on a few occasions that carefully dried precipitated acetamide suddenly became normal. It is suggested tentatively that these observations are due to tautomeric shifts of acetamide. If the extreme effect of acetamide on serum proteins (corresponding to the minimum of the melostagmin curves) is observed at the limit of integral combination between serum proteins and acetamide,

one arrives at a protein:acetamide molar ratio of about of 10^{-5} . Even on the assumption that only the α -lipoglobulins of the serum (226) react, the ratio is still of the order of 10^{-3} - 10^{-4} . This leads to the hypothesis that only about 0.1 - 0.01% of the acetamide takes part in the reaction. Attempts to clarify this matter by meiostagmin reactions with various fractions of acetamide have been unsuccessful so far.

Meiostagmin reactions on albumin or globulin alone gave normal tests. The same applies to mixtures of albumin and globulin, although an excess of globulin appears to shift the test curve slightly towards the habitus displayed by cancer sera. Cancer sera after extraction with ether behave in the modified meiostagmin test like mixtures of albumin and globulin. Reincorporation of the ether extract does not restore the typical cancer curve although it modifies the normal curves towards the cancer one. These relations are illustrated in Figs. 1 and 2.

Total protein and albumin:globulin assays have been carried out on a few cancer and other sera. The average total protein for 35 cancer sera was 6.86% and for 13 other sera 7.56% (7 normals 7.45%, 6 non-cancer complaints 7.67%). The average albumin:globulin ratios were 0.67 for cancer sera and 0.86 for others. These figures are consistently higher than checking assays carried out by Dr. M.P.K. Shoobridge at the General Hospital, Launceston who, however, did not standardize his results against macro-Kjeldahl determinations as in our case. In spite of the considerable differences between the average values for cancer and other sera, these assays have no diagnostic value since the variations in the cancer and non-cancer series overlap and the

different averages are due to a few extreme cases.

The use of 1-phenyl-3,5-dimethyl-1,2,4-triazole (abbreviated as "triazole" on the curves) in molistagnin reactions gives considerable qualitative and quantitative differences between cancer and non-cancer sera. Figs. 3 and 4 illustrate the molistagnin reaction on the same pair of sera with 5% acetamide and 1-phenyl-3,5-dimethyl-1,2,4-triazole. It is admitted, however, that more experimental work will be needed to confirm the statistical significance of these differences. A rare case of thyreotoxicosis gave a curve with a slight downward slope after the "parallel section" of the curve and thus appeared to be intermediate between cancer and non-cancer cases from the point of view of this test. Fig. 5 shows some molistagnin reactions of serum proteins with 5% 1-phenyl-3,5-dimethyl-1,2,4-triazole. Further data will be required to elucidate the nature of aqueous protein-triazole systems but the few available data suggest that the components interact.

From a theoretical point of view 1-phenyl-3,5-dimethyl-1,2,4-triazole is not an ideal reagent in diagnostic tests based on surface tension measurements. Only freshly recrystallized material in fresh solutions gives results which can be duplicated. In older material the surface tension does not come to equilibrium, presumably owing to continuous slow changes in the degree of association in water. Even fresh material does not follow Szyszkowski's equation (227) in concentrations exceeding 4-5%. At lower concentrations the Szyszkowski constants are $A = 0.13$, $B = 0.25$ (calculated by P. Dunn). The surface tension versus concentration curve for 1-phenyl-3,5-dimethyl-1,2,4-triazole is

shown in Fig. 6.

The Szyszkowski curve for thymus nucleic acid in a borate buffer of pH 7.89 is shown in Fig. 7. The slope of the curve does not permit the calculation of significant Szyszkowski constants. Melostagmin curves of the same nucleic acid with acetamide and 1-phenyl-3,5-dimethyl-1,2,4-triazole are shown in Fig. 8 and indicate some measure of chemical interaction between the nucleic acid and the two investigated melostagmin reagents. As in the case of the melostagmin reaction with proteins, a more definite discussion requires more information on the homogeneity and reactive fractions of the components of the system. It is hoped to report on these matters in the near future.

Finally, another series of incomplete experiments is noted owing to the potential importance of the preliminary findings. Acetamide seems to protect serum proteins against coagulation by heat. This effect is more pronounced with non-cancer sera. A similar effect was noted with 1-phenyl-3,5-dimethyl-1,2,4-triazole in which case, however, the conditions of the effect appear to be very involved. Transient precipitation may occur even in the cold at high serum concentrations. The protective effect is apparent on either side of the concentration corresponding to the surface tension minimum but at that concentration coagulation or precipitation by heat is enhanced. It was hoped to evolve a test similar to the iodoacetic acid test of Huggins, Miller and Jensen (228) on these observations but these attempts have not been successful so far.

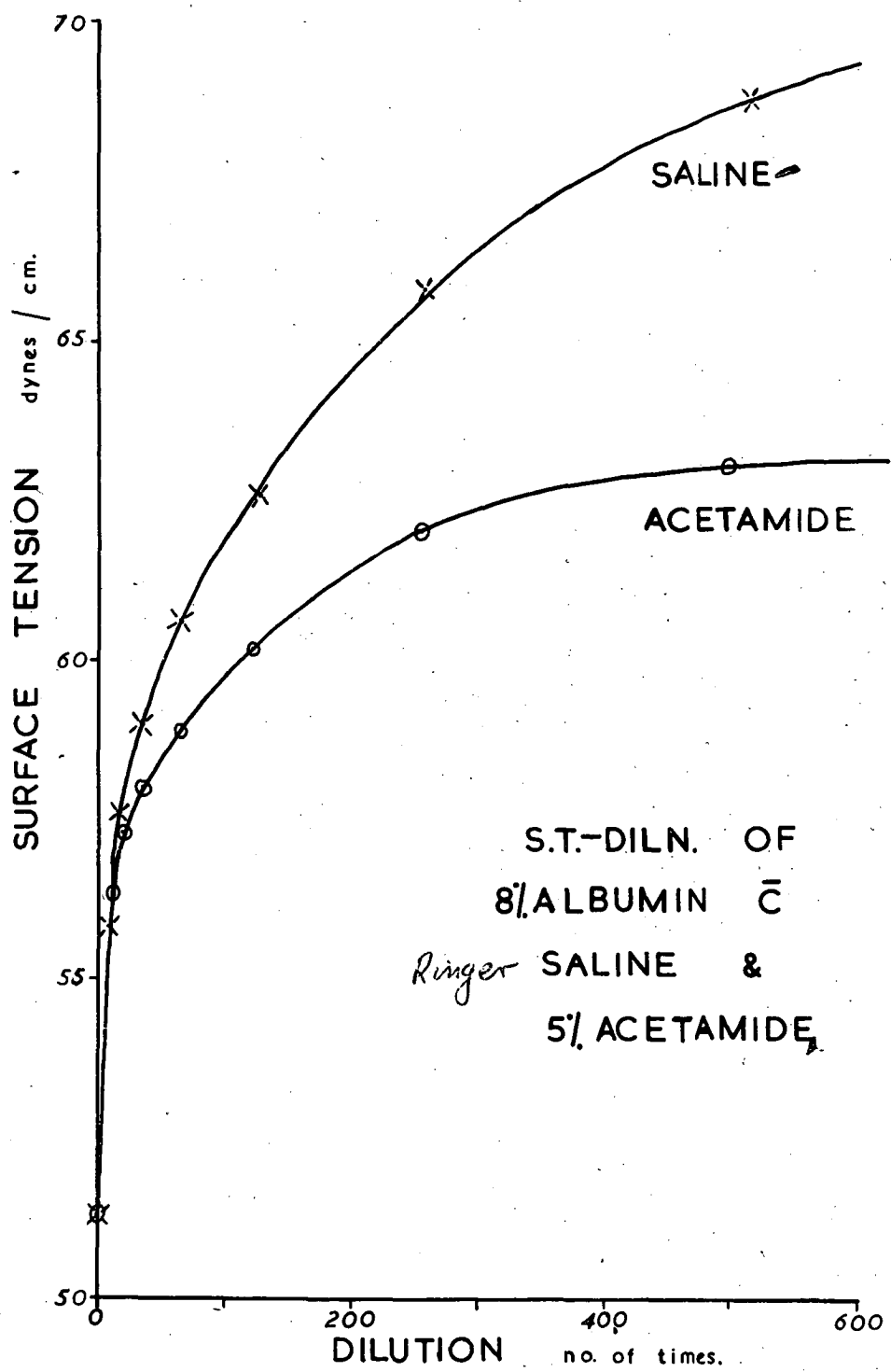


Fig. 1.

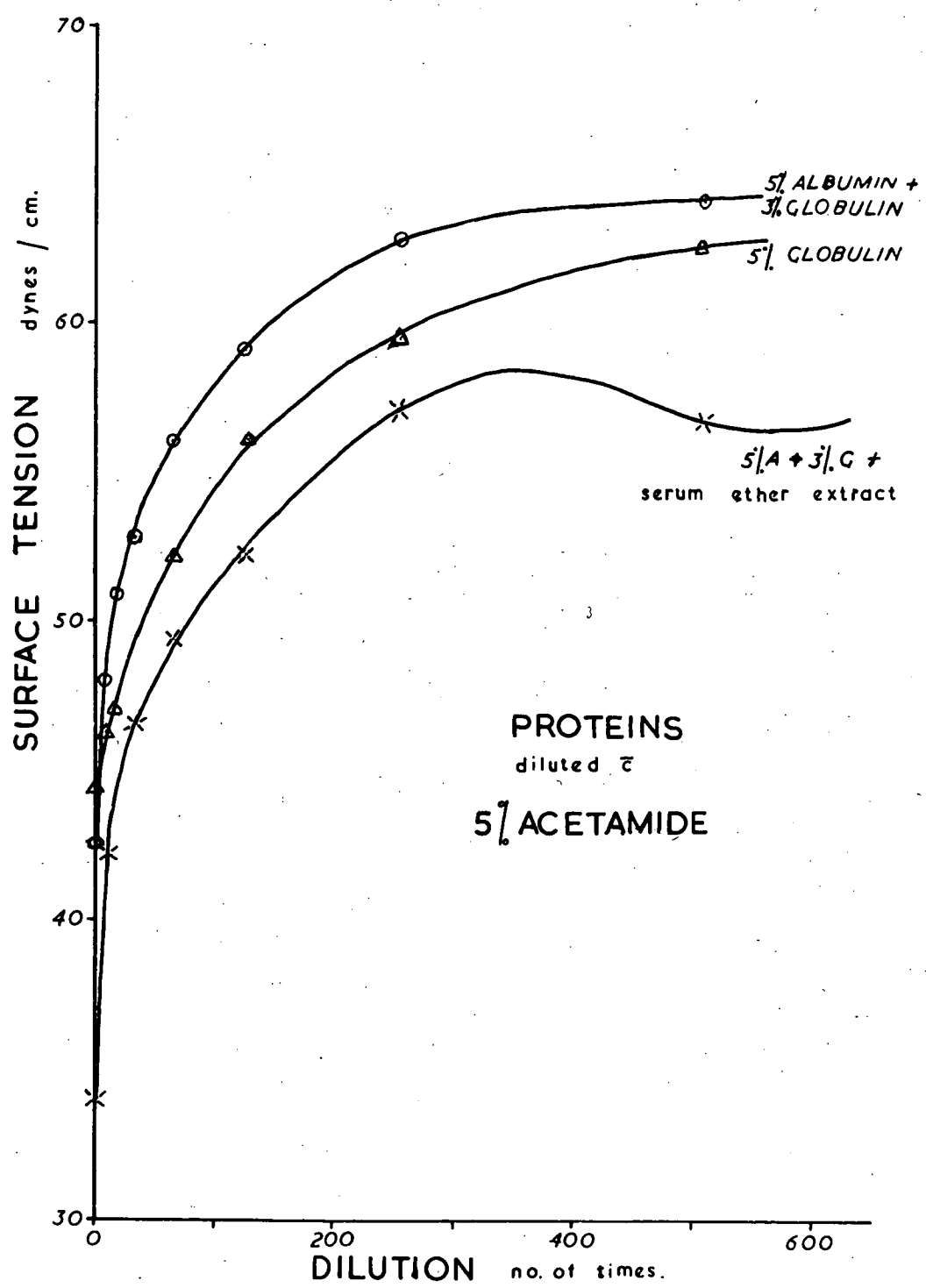


Fig. 2.

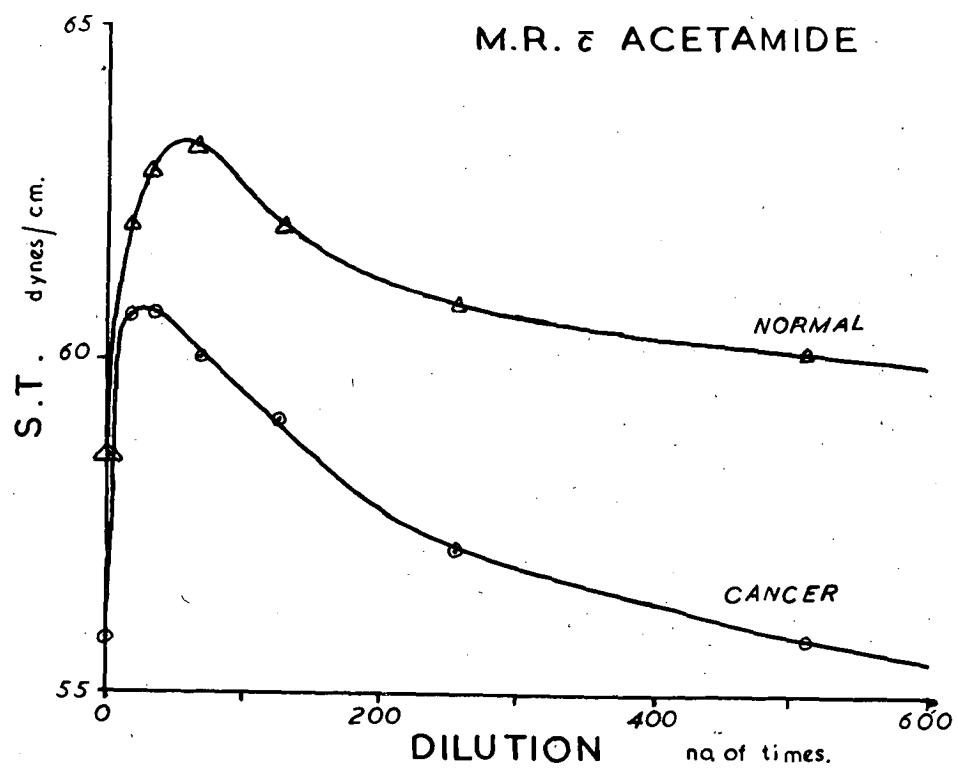


Fig. 3

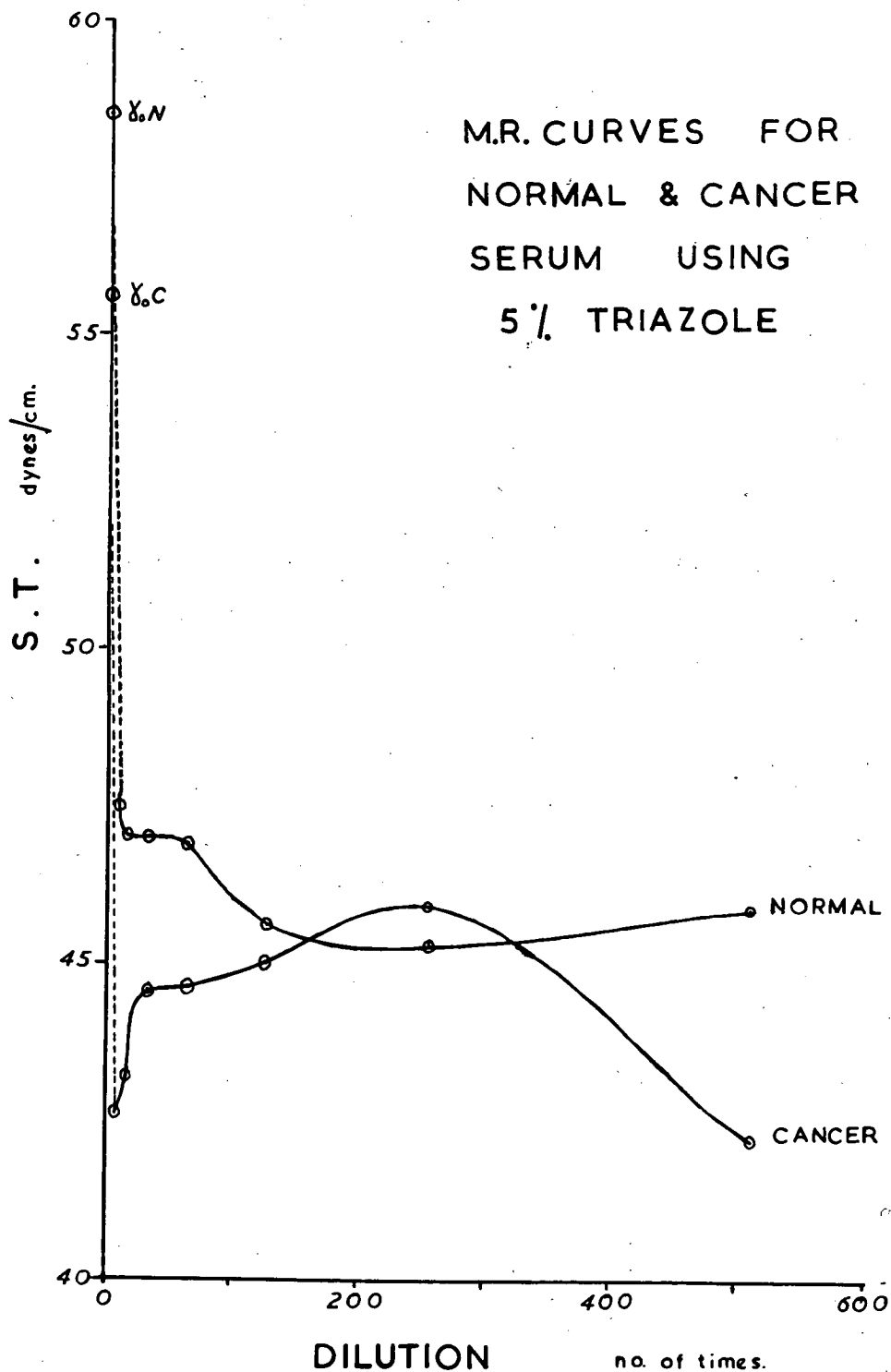


Fig. 4.

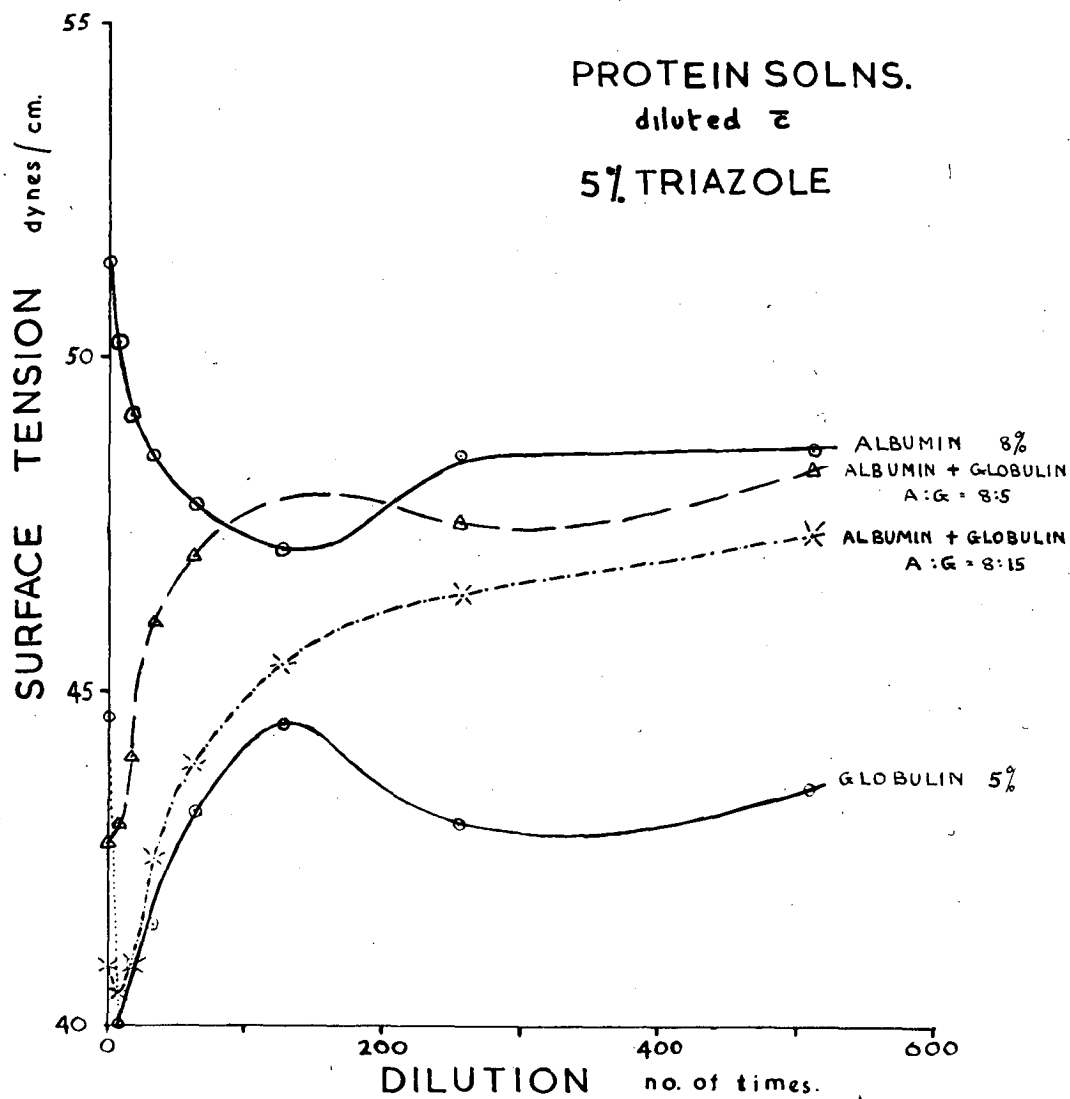


Fig. 5.

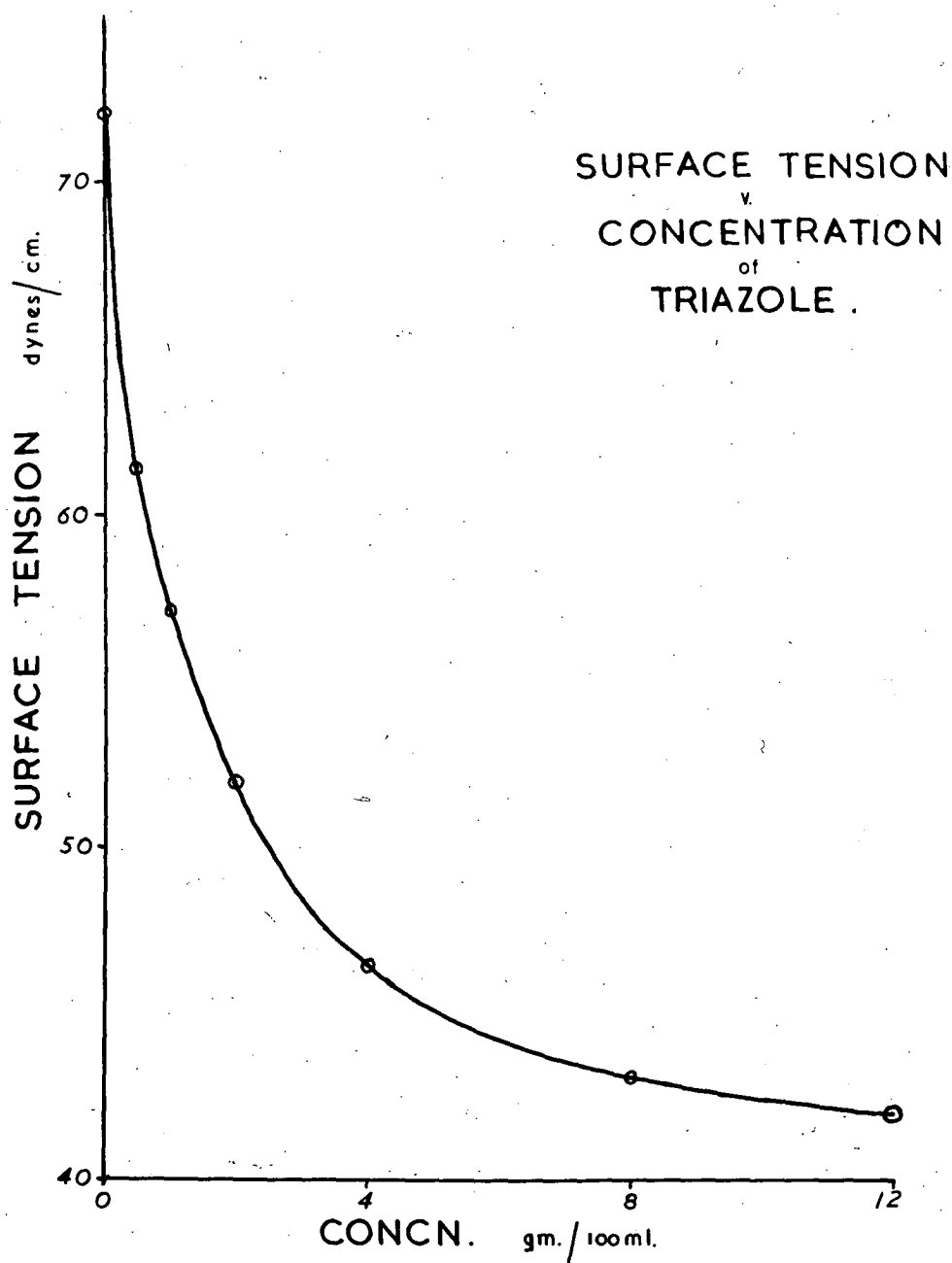


Fig. 6

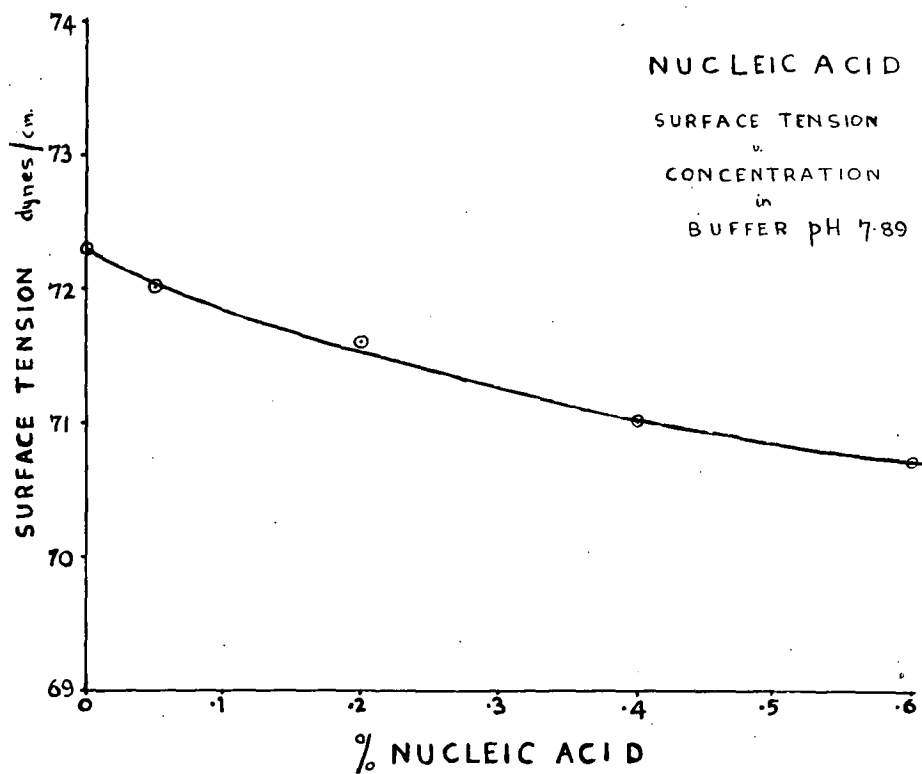


Fig. 7.

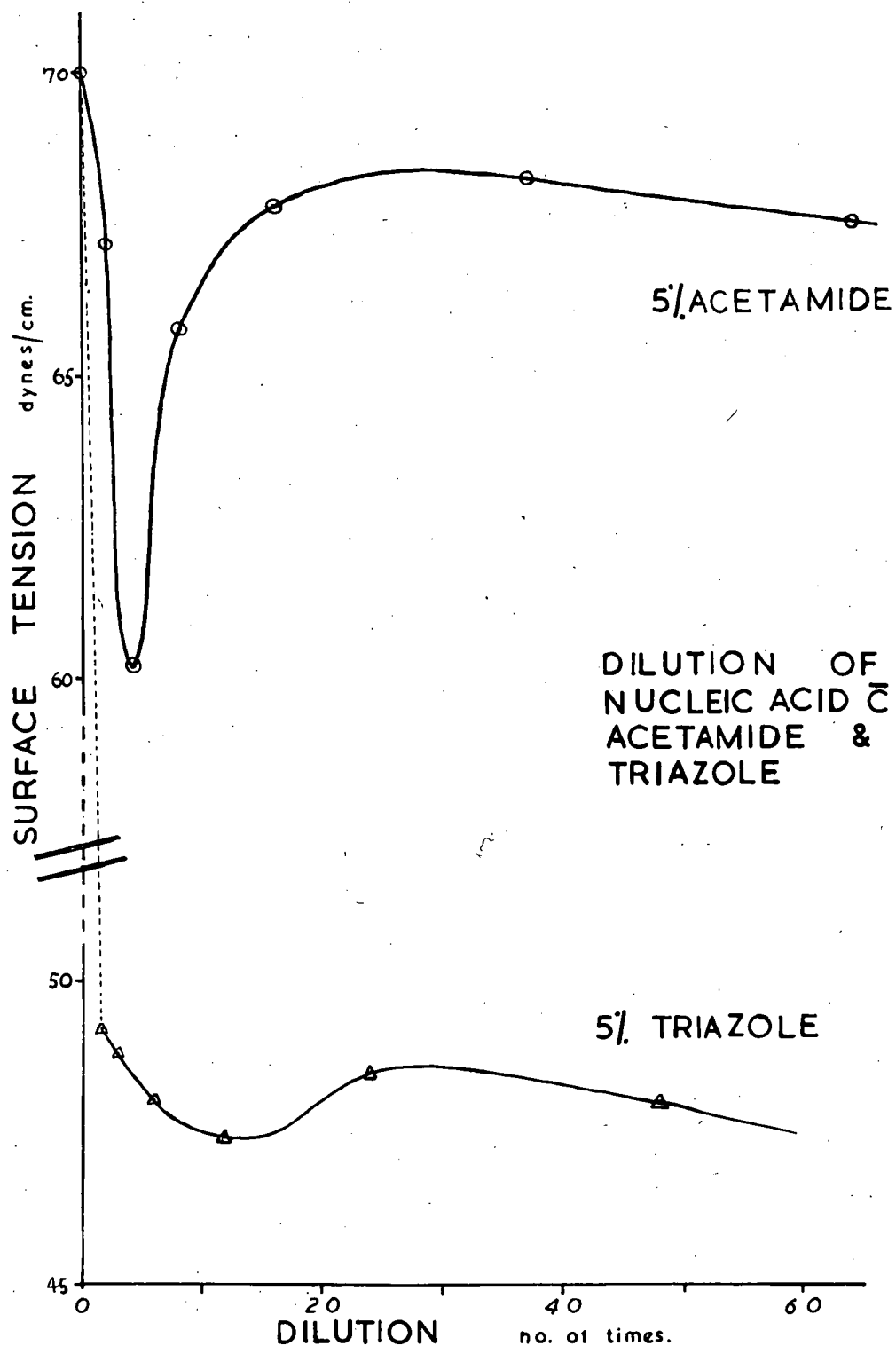


Fig. 8.

ADDENDA

to p.1 : The following papers have been accepted for publication :
J.B.P. - P.L.Tardew (Analytical Chemistry)
J.B.P. -T.E.Spotswood, 2 papers (Rec.trav.chim.Pays-Bas).

For the paper by J.B.P. -P.Dunn see correction under
References 4) .

- to pp.5-7: Owing to different editorial requests the author had to depart from the suggested nomenclature in some of the attached papers .Reference to II.4 (pp. 74-80) may strengthen arguments in favour of the term diacylimine although it is possible that an eventual proof of the existence of form 4 (p. 77 , Table 4) as a substance of some stability will restrict this term to this form only .
- to p. 49 : n-Butyryl benzamide is not the only diacylimine with a double melting point .In some preparations of acetyl propionamide the normal (m.p. 86°) substance is accompanied by a higher melting substance (m.p. 116°) . The lower melting substance does not depress the melting point of the higher melting substance . This matter is investigated at present with Dr.A.Komzak.
- to p. 53 : Di-n-butyrimide requires 8.92% N , found 8.90% (P.D.)
- to p. 57 : Acetyl butyranilide requires 6.83% N , found 6.62% (P.D.)
- to p. 58 : The recrystallization of N,N-diacetyl 2-naphthylamine is difficult since the substance is too soluble in the usual solvents . Good crystals were obtained by recrystallizing from kerosene which has been washed with sulphuric acid , then dilute sodium hydroxide , dried over calcium chloride and distilled. A fraction going over between 180 - 190 ° was used for recrystallization. The crystals may be freed from the residual traces of solvent in vacuum (drying pistol) and storage in vacuum over paraffin chips . The substance recrystallized in this manner has still a faint yellow colour . The melting point is 67 - 68° .

to p. 80 : Dr.H.W.Thompson , F.R.S. has agreed to measure the the infra-red absorption spectra of a number of diacylimines , benzylidene and furylidene bisamides and triazoles prepared by the author and his students. This work will be carried out early in 1951 .

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Owing to delays of mails , some publications were received too late to receive attention in this Thesis .

Recent papers by Emery and Gold (229 - 231) support the postulated equation b8) (p.36) and may be regarded as models for the kinetic extensions of the work reported in this Thesis .

The abnormal reactions of amides with Grignard compounds (232) could be explained by assuming that such amides react in the imidol form , or , in the case of N,N-disubstituted amides , in the enolized form $\text{RCH:C(OH)NR}'_2$ (cf.p. 79) .

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COLORED FERRIC ASSAY OF DIACYLAMIDES

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Summary: Diacylamides may be assayed by converting them to hydroxamic acids and measuring the optical density after adding ferric chloride. Readily soluble diacylamides, if present in biological systems, would interfere to some extent with the acetyl phosphate assay of Lipmann and Tuttle. Following the Lipmann - Tuttle procedure but using more alkali for shorter reaction times diacylamides may be determined with an error of $\pm 4\%$. The assay is not suitable for N-formyl amides rapidly hydrolyzed diacylamides like acetyl chloroacetamide and acetyl phosphamide.

Lipmann and Tuttle (5) have developed a procedure to assay acyl phosphates by converting the organic acyl radicals to hydroxamic acids which give intense coloration with ferric chloride. Since purely organic anhydrides or acyl chlorides are not likely to occur under biological conditions and esters or amides do not react under the experimental conditions, the method is claimed to be specific for acyl phosphates in biological materials. If it possible, however, that diacylamides of the general formula $RCO.NH.R'.COH$ may occur in biological systems. The Lipmann-Tuttle assay was applied to a number of representative compounds of this class. Dibenzamide (1), formyl benzamide (4), diacetamide (10) and other diacylamides with $R' = H$ (7) were prepared by previously

described methods. Other diacylamidos were prepared by the acylation of the appropriate amines or amides with anhydrides in the presence of acyl halides or thionyl chloride in catalytic amounts (3). Other reagents were commercial products of analytical quality.

The reagent solutions were those recommended by Lipmann and Tuttle. They were prepared fresh and used in the quantities and order as suggested by the originators of the method. The colors were compared with a Hilger Spekker Absorptionmeter using 1 cm cells Ilford 608 gelatine filters and a mixture of the reagents only as reference. This gave convenient readings for the assay of succinic anhydride or acetyl phosphate in concentrations ranging from 0.025M to 0.100M with an error of $\pm 4\%$. This procedure was not suitable for diacylamides since some of them were not sufficiently soluble and even diacetamide gave low density readings with an error of $\pm 18\%$. Table 1. refers to average values from four measurements each, with errors indicated above.

TABLE 1.

| Molar concentration | Density | |
|---------------------|--------------------|-------------|
| | Succinic Anhydride | Diacetamide |
| 0.100 | 1.18 | 0.13 |
| 0.075 | 0.89 | 0.11 |
| 0.050 | 0.59 | 0.09 |
| 0.025 | 0.30 | 0.05 |

Better results were obtained by increasing the alkalinity of the reaction mixture and limiting the reaction time to 2 minutes. In this modified procedure 1 cc each of 4M hydroxylamine hydrochloride solution and 3.5M sodium hydroxide are mixed and 1.8 cc of the recommended acetate buffer is added. The solution to be analyzed (2 cc.) followed by another 0.2 cc. of the 3.5M sodium hydroxide are added in this order and the mixture is allowed to stand for 2 minutes at room temperature (18-20°). The subsequent operations are those recommended by Lipmann and Tuttle. Ilford 604 gelatine filters were used in the modified procedure. Dibenzamide is not sufficiently soluble in water and was used dissolved in 1% sodium hydroxide. Other relatively insoluble diacylamides like acetyl and propionyl benzamide hydrolyze too rapidly in alkaline solutions. In such cases the assays had to be restricted to lower concentrations in water only. Although N-methyldiacetamide is very soluble in water, such solutions hydrolyze rapidly and the assay must be carried out without delay. Even then the assay has an error of $\pm 7\%$ against $\pm 4\%$ in other cases with the exception of acetyl chloroacetamide which is assayed with an error of $\pm 22\%$. The method is not suitable for the assay of N-formyl amides since the colour fades within a few seconds. The reaction therefore proceeds as $\text{PhCONHCHO} + \text{NH}_2\text{OH} \longrightarrow \text{PhCONH}_2 + \text{HC}(:\text{O})\text{NHOH}$ in agreement with previous theories (7). Hydantein, N-methyl-N'-acetylurea, succinimide and acetanidine gave little or no color. Acetamide in a 5% (0.847M) solution gave an intensity of 0.27 ± 0.02 corresponding to that given by approximately 0.0025M solutions of diacylamides. The N-bromo and N-bromo-magnesium

derivatives of diacetamide (6) gave the color test which, however, was not suitable for quantitative purposes owing to the instability of these compounds under the experimental conditions. The same applies to a lesser extent to acetyl chloroacetamide but the colorimetric assay of some halogenated diacylamides could be supplemented by fluorimetric or spectrographic methods (8, 9). Esters interfere in the presence of excess alkali (11) although this is not the case in the original method of Lipmann and Tuttle. However the removal of esters from solutions containing acyl phosphates is difficult or impossible, whereas the separation of esters from most diacylamides is comparatively simple.

Average results of six assays (four assays only for acetyl chloroacetamide) are shown in Table 2. The errors indicated above refer to concentrations from 0.0025M to 0.0100 M. At 0.0010M concentration the errors are estimated at $\pm 9-10\%$. The density versus concentration curves may be regarded as linear within the stated range of errors and may be used for the rapid determination of small amounts of diacylamides. The assays may be duplicated with an error of $\pm 2\%$ only if succinic anhydride is used in the reference cell as in the original Lipmann-Tuttle procedure. Diacetamide is unsatisfactory as a reference in the original but useful in the modified methods.

TABLE 2.

| Compound | Density | | | | |
|------------------------|---------|---------|---------|---------|---------|
| | 0.0100M | 0.0075M | 0.0050M | 0.0025M | 0.0010M |
| Succinic anhydride | 1.18 | 0.89 | 0.59 | 0.30 | 0.14 |
| Diacetamide | 1.33 | 0.99 | 0.65 | 0.33 | 0.15 |
| N-methyldiacetamide | 1.07 | 0.73 | 0.50 | 0.26 | 0.12 |
| Diacetanilide | 1.33 | 0.97 | 0.65 | 0.30 | 0.14 |
| Acetyl propionamide | 1.29 | 0.95 | 0.64 | 0.32 | 0.15 |
| Dipropionamide | 1.39 | 1.02 | 0.69 | 0.34 | 0.14 |
| Acetyl benzamide | insol. | insol. | 0.69 | 0.36 | 0.16 |
| Propionyl benzamide | insol. | insol. | 0.72 | 0.35 | 0.16 |
| Dibenzamide | 1.75 | 1.25 | 0.82 | 0.41 | 0.19 |
| Acetyl chloroacetamide | 1.10 | 0.65 | 0.52 | 0.27 | |

Analytically pure disilver acetyl phosphamide has been prepared by dehydrating and acylating monoammonium phosphate with acetic anhydride in excess in the presence of thionyl or acetyl chloride and precipitation from an aqueous solution with silver nitrate (2). Like benzoyl phosphamide (12), acetyl phosphamide appears to be relatively stable in aqueous solution. Removal of the silver by thioacetamide resulted in solutions from which the original silver compound salt could be regenerated with a loss of 71-86%. Such silver-free solutions could not be used for the assay because of a slowly fading green colour due to residual thioacetamide in the solution.

Fresh extracts of disilver acetyl phosphamide with a slight excess of 10% sodium chloride solution did not give the test either by the original or the modified Lipmann-Tuttle procedure. A faint colour appeared occasionally after standing for a few hours. This suggests that the cleavage of acetyl phosphamide by hydroxylamine yields acetamide and phosphohydroxylamine probably followed by the rapid hydrolysis of the latter.

The original and modified test has been applied to cancer, diabetes, pregnancy and normal human serum and to albumin, globulin, euglobulin and pseudoglobulin fractions of such sera. As a rule the test was negative but a few fresh, defibrinated sera from diabetic patients gave a faint purple color which was retained in the centrifuged precipitated proteins.

The authors wish to acknowledge a grant from Mr. E.J. Hallstrom and thank Messrs. G.C. Bratt and P. Dunn for pure preparations of some of the investigated compounds.

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ANIDES VI. : Studies on Acetamide and Diacetimide.

by P. Dunn and J.B. Polya

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SUMMARY: Diacetimide is best purified by recrystallization from petroleum ether. The melting point curve of mixtures of acetamide and diacetimide establishes the existence of $\text{AcNH}_2 \cdot \text{Ac}_2\text{NH}$ and may be used for the approximate evaluation of the purity of diacetimide preparations. Aqueous solutions of acetic acid, acetamide and diacetimide obey Szyszkowski's equation. Aqueous solutions of diacetimide are less stable than those of acetamide and decompose with a monomolecular velocity constant.

The present paper reports chemical studies in connection with biochemical investigations on surface tension buffering in human sera (1). Early experiments indicated that, while acetamide solutions gave consistent results, it was impossible to duplicate results with satisfactory accuracy when using diacetimide prepared by the improved Hentschel method (2). In this method acetamide and diacetimide are separated by treating their ethereal solution with dry hydrogen chloride which precipitates bisacetamide hydrochloride and leaves diacetimide in solution. Simple as the method is in principle, considerable experience is required to obtain neutral diacetimide free from acetamide. The removal of hydrogen chloride is effected by potassium carbonate and barium carbonate but this process is not fully effective unless a trace of water is present. In the latter case, however, some of the diacetimide will be hydrolysed with the formation of acetamide. Even the best preparations, as judged by

analysis and melting point, may retain traces of hydrogen chloride which reduces the stability of aqueous solutions. This effect is easily tested by measuring the surface tension of diacetimide solutions. In 5% aqueous solutions of pure acetamide and diacetimide have surface tensions of 70.1 and 67.8 dyne/cm respectively. (All surface tension data in this paper refer to dyne/cm units at 18 C° and are denoted by the symbol γ). If acetic acid is present the surface tension may sink as low as 49 dyne/cm, although acetic acid is not solely responsible for this effect.

Systematic recrystallization from petroleum ether instead of Strecker's hydrogen chloride method reduces the yield but gives very pure and stable diacetimide. Petroleum ether boiling between 60-80 C° is the most suitable for this purpose. Higher boiling fractions dissolve too much acetamide and the solubility of diacetimide is too low in lower boiling fractions. This method of purification has been applied with success to other diacylimines, such as acetyl propionamide, monochlorodiacetimide and acetyl benzamide (3).

Melting points of pure acetamide and diacetimide were plotted against percentage composition. Eutectic minima were noted at compositions corresponding closely to molecular acetamide-diacetimide ratios of 1:2 and 2:1. A maximum appeared at the molecular composition of 1:1 (Figure 1) thus indicating the existence of $\text{AcNH}_2 \cdot \text{Ac}_2\text{NH}$ as a chemical individual (4,5).

Molecular weight determinations could not be carried out since the compound dissociates readily in solvents. The series of fused mixtures was dissolved in distilled water to give 5% solutions. The surface tension versus composition curve was plotted for this series.

The curve was irregular and could not be duplicated with precision but it seemed to follow the melting point curve. The results of these experiments are shown in Table 1 which may be used for the approximate determination of the purity of diacetimide preparations.

TABLE 1.

| %Ac ₂ NH in mixture | %AcNH ₂ in mixture | Melting point °C. | γ of 5% Soln. in distilled water at 18°C. dyne/cm. |
|-----------------------------------|----------------------------------|----------------------|---|
| 0 | 100 | 79 | 70.2 |
| 15 | 85 | 71.5 | 69.3 |
| 22.5 | 77.5 | 67.0 | 69.2 |
| 30 | 70 | 64.5 | 68.5 |
| 37.5 | 62.5 | 61.3 | 68.5 |
| 45 | 55 | 60.0 | 68.1 |
| 50 | 50 | 61.0 | 68.0 |
| 54 | 46 | 63.0 | 67.7 |
| 60 | 40 | 65.0 | 68.0 |
| 63.1 | 36.9 | 65.7 | 68.2 |
| 70 | 30 | 63.0 | 67.8 |
| 75 | 25 | 61.0 | 67.6 |
| 77.2 | 22.8 | 59.0 | 67.8 |
| 82.5 | 17.5 | 61.0 | 67.8 |
| 84 | 16 | 62.0 | 67.8 |
| 90 | 10 | 69.0 | 67.7 |
| 100 | 0 | 79.0 | 67.8 |

When 5% solutions of acetamide and diacetimide were mixed in various proportions, the surface tension versus composition curve showed a regular, slightly hyperbolic habitus without maxima and

minimo. It appears therefore that the acetamide-diacyetamide complex is not formed in water although decomposition of the complex, formed during fusion, is not instantaneous. These experiments were repeated in solutions with a total concentration of 10%. In a further series the experiments were repeated with the addition of acetic acid in quantities equimolecular with acetamide to the solutions. This was done to study the behaviour of decomposing solutions of diacyetamide in which acetamide and acetic acid are formed in equimolecular quantities. If the surface tensions of 5% and 10% solutions containing acetamide and diacyetamide only are designated as δ_{5i} and δ_{10i} and corresponding solutions containing acetic acid in equimolecular amounts with acetamide δ_{5j} and δ_{10j} for corresponding acetamide-diacyetamide compositions, it will be seen that $\delta_{10i} - \delta_{10j} / \delta_{5i} - \delta_{5j} = K$ is approximately constant. (Figure 2. and Table 2.).

TABLE 2.

| Acetamide in Mixture 1 | δ_{10i} | δ_{10j} | $\delta_{10i} - \delta_{10j}$ | δ_{5i} | δ_{5j} | $\delta_{5i} - \delta_{5j}$ | K |
|------------------------------|----------------|----------------|-------------------------------|---------------|---------------|-----------------------------|------|
| 0 | 68.5 | 55.0 | 13.5 | 70.1 | 61.0 | 8.9 | 1.50 |
| 10 | 68.2 | 56.1 | 12.1 | 69.7 | 61.6 | 8.1 | 1.50 |
| 20 | 67.8 | 57.1 | 10.7 | 69.5 | 62.1 | 7.4 | 1.49 |
| 30 | 67.3 | 57.8 | 9.5 | 69.2 | 62.8 | 6.4 | 1.49 |
| 40 | 66.9 | 58.5 | 8.4 | 69.0 | 63.4 | 5.6 | 1.50 |
| 50 | 66.4 | 59.4 | 7.0 | 68.7 | 64.1 | 4.6 | 1.52 |
| 60 | 66.0 | 60.4 | 5.6 | 68.5 | 64.9 | 3.6 | 1.56 |
| 70 | 65.6 | 61.4 | 4.2 | 68.3 | 65.8 | 2.5 | 1.70 |
| 80 | 65.3 | 62.6 | 2.7 | 68.1 | 66.5 | 1.6 | 1.69 |
| 90 | 65.0 | 63.7 | 1.3 | 68.0 | 67.2 | 0.8 | 1.62 |
| 100 | 64.8 | 64.8 | 0. | 67.8 | 67.8 | 0. | - |

The surface tensions of acetic acid, acetamide and diacyetamide in water were measured at different dilutions (Figure 3.).

Writing γ_0 for the surface tension of water, γ for that of aqueous solutions C for the concentration of the solute (moles/litre) with A and B for constants, Szyszkowski's equation (6) states

$$\frac{\gamma_0 - \gamma}{\gamma_0} = B \log_{10} \left(\frac{C}{A} + 1 \right) \quad \text{.....1)}$$

The constants A and B have been determined graphically for acetic acid, acetamide and diacetimide and are shown in Table 3. These constants have been determined for acetic acid by Traube (7) whose results we were able to confirm. Tables 4, 5 and 6 show the good agreement between observed values and those calculated from the constants appearing in Table 3.

TABLE 3.

| | A | B |
|--------------------|------|------|
| AcOH | 0.37 | 0.32 |
| AcNH ₂ | 0.74 | 0.12 |
| Ac ₂ NH | 0.64 | 0.29 |

TABLE 4.

Acetic acid in water T = 18°C

| C Mols/Litre | γ (obs) | γ (calc.) | % error |
|--------------|----------------|------------------|---------|
| 0 | 73.0 | - | - |
| 0.083 | 70.7 | 70.9 | 0.28 |
| 0.167 | 68.9 | 69.1 | 0.29 |
| 0.333 | 66.2 | 66.4 | 0.30 |
| 0.500 | 64.0 | 64.2 | 0.31 |
| 0.667 | 62.3 | 62.5 | 0.32 |
| 0.833 | 61.0 | 61.0 | 0.00 |
| 1.000 | 59.7 | 59.7 | 0.00 |
| 1.333 | 57.5 | 57.4 | -0.17 |
| 1.667 | 55.3 | 55.6 | 0.54 |

TABLE 5.

Acetamide in Water T = 18°

| C Mols/Litre | χ (obs) | χ (calc.) | % error |
|--------------|--------------|----------------|---------|
| 0 | 73.0 | - | - |
| 0.106 | 72.4 | 72.5 | +0.14 |
| 0.212 | 72.0 | 72.0 | 0.00 |
| 0.424 | 71.2 | 71.3 | +0.14 |
| 0.847 | 70.1 | 70.1 | 0.00 |
| 1.271 | 69.2 | 69.1 | -0.14 |
| 1.695 | 68.4 | 68.4 | 0.00 |
| 2.543 | 67.2 | 67.3 | +0.14 |

TABLE 6.

Diacetamide in water T = 18°

| C Mols/Litre | χ (obs) | χ (calc.) | % error |
|--------------|--------------|----------------|---------|
| 0 | 73.0 | - | - |
| 0.061 | 72.1 | 72.2 | +0.14 |
| 0.124 | 71.4 | 71.4 | 0.00 |
| 0.248 | 69.8 | 69.9 | +0.14 |
| 0.495 | 67.8 | 67.8 | 0.00 |
| 0.743 | 66.0 | 66.0 | 0.00 |
| 0.990 | 64.5 | 64.5 | 0.00 |

No significant results could be obtained with the following compounds at maximum concentrations shown in brackets: urea (10%) ammonium acetate (20%) and acetyl propionamide (0.5%). In the latter case the slight solubility and moderate surface activity of the compound presented a difficulty which could not be overcome with our equipment. The same applies to surface tension measurements of mixed acetamide-diacetamide solutions at total concentration lower than 2%. Bisacetamide hydrochloride in a 5% aqueous solutions had a surface tension of 68.5 dyne/cm. Owing to the rapid dissociation of this compound in water, this figure is not significant in itself except for the fact that the presence of traces of bisacetamide hydrochloride may be disregarded as one of the factors responsible for abnormally low surface tensions of diacetamide solutions.

When the material reported so far is viewed as a whole, it will be seen that our initial problem remains unsolved. The decomposition of diacetamide in water, as far as it is known, yields acetic acid, acetamide and (in neutral solutions) ammonium acetate. Low surface tensions of around 50 dyne/cm in 5% diacetamide solutions cannot be traced to any of these compounds since even the complete hydrolysis of such a solution to acetic acid and acetamide would yield an approximately half-molar solution of acetic acid with a surface tension of 64.0 dyne/cm. and an acetamide solution of similar molarity with a surface tension of 70.1 dyne/cm. The surface tension of an aqueous solution containing acetic acid and acetamide both at half-molar concentrations is 61.0 dyne/cm, that is considerably lower than that of either constituent or any linear sum of the

constituent surface tensions. To explain the effect one must assume the existence of molecular adducts with decreased solubilities in water. In the case of the pair acetic acid acetamide this view is strengthened by the work of Luck and his collaborators on combination between acids and serum proteins (3). In pairs involving diacetamide this theory is supported by an observation which, although not fully elucidated, is surprising enough. Concentrated solutions of diacetamide become cloudy on standing and small amounts of an insoluble material precipitate. The process is speeded up by adding potassium carbonate, even to dilute solutions of diacetamide, so as to bring the pH to about 9. Yields are poor by either method and do not exceed 0.3%. The material is colourless, amorphous or microcrystalline, insoluble in water, alcohol, ether, acetone, chloroform, dilute acids and alkalis but somewhat soluble in glacial acetic acid. The substance has an unsharp melting point around 360 C° and contains nitrogen. The Murexide test and tests for known xanthine and pyrimidine compounds were negative.

All this weakens somewhat the validity of assuming the single equation



as the governing principle of the decomposition of diacetamide in aqueous solutions. It will be seen, however, that Equation 2) may be used for the approximate description of the decomposition of aqueous diacetamide solutions on standing. Acetamide in 0.1, 1, 5 and 10% solutions did not show significant pH changes over 4 days hence it must be regarded as very stable. Surface tension measurements in biological media, however, revealed that this stability

is apparent only and does not extend to more than a day or so (1). The pH changes of 1% and 5% solutions of diacetimide in water were observed over 480 hours at 18 C°. The pH values were converted to the corresponding concentrations of acetic acid and thus the degree of the hydrolysis of diacetimide and the rate constant for a monomolecular reaction were calculated. In spite of such an imperfect procedure the velocity constant k was found to equal 1.66×10^{-4} in both experiments (Figures 4. and 5., Tables 7. and 8.) A colorimetric assay of diacetimide is being investigated at present. This will permit more detailed kinetic studies in the near future.

TABLE 7.

| Time (hrs.) | pH | AcOH Mols/litre | % Dissociation of Ac ₂ H ₄ in 5% soln. | k (hrs. ⁻¹) |
|----------------|------|--------------------|--|-------------------------|
| 0.05 | 5.08 | 0.000031 | 0.006 | - |
| 0.5 | 4.80 | 0.000050 | 0.010 | - |
| 1.0 | 4.60 | 0.000073 | 0.015 | - |
| 2.0 | 4.35 | 0.00020 | 0.04 | - |
| 3.0 | 4.23 | 0.00031 | 0.06 | - |
| 4.0 | 4.15 | 0.00040 | 0.08 | - |
| 5.0 | 4.07 | 0.00051 | 0.10 | - |
| 6.0 | 4.02 | 0.00060 | 0.12 | - |
| 6.6 | 4.00 | 0.00065 | 0.13 | - |
| 7.0 | 3.98 | 0.00070 | 0.14 | - |
| 8.0 | 3.95 | 0.00080 | 0.16 | 2.01×10^{-4} |
| 30.0 | 3.68 | 0.0029 | 0.60 | 2.00×10^{-4} |
| 60.0 | 3.50 | 0.0053 | 1.1 | 1.84×10^{-4} |
| 90.0 | 3.40 | 0.0080 | 1.6 | 1.79×10^{-4} |
| 120.0 | 3.33 | 0.011 | 2.2 | 1.85×10^{-4} |
| 150.0 | 3.28 | 0.013 | 2.6 | 1.76×10^{-4} |
| 180.0 | 3.25 | 0.014 | 2.8 | 1.58×10^{-4} |
| 240.0 | 3.19 | 0.017 | 3.4 | 1.44×10^{-4} |
| 300.0 | 3.14 | 0.020 | 4.0 | 1.36×10^{-4} |
| 360.0 | 3.09 | 0.025 | 5.0 | 1.43×10^{-4} |
| 420.0 | 3.04 | 0.032 | 6.4 | 1.58×10^{-4} |
| 480.0 | 3.01 | 0.037 | 7.4 | 1.60×10^{-4} |

The average value of k for all times greater or equal to 30.0 hours is 1.66×10^{-4} hrs. ⁻¹.

TABLE 8.

| Time (hrs.) | pH | AcOH Mols./litre | % Dissociation of $\text{Ac}_{2\text{III}}$ in 1% soln. | k (hrs. $^{-1}$) |
|----------------|------|---------------------|---|-----------------------|
| 0.05 | 5.77 | 0 | 0 | - |
| 0.5 | 5.60 | 0.000010 | 0.010 | - |
| 1.0 | 5.46 | 0.000014 | 0.014 | - |
| 2.0 | 5.20 | 0.000025 | 0.025 | - |
| 3.0 | 4.97 | 0.000037 | 0.037 | - |
| 4.0 | 4.83 | 0.000047 | 0.047 | - |
| 5.0 | 4.70 | 0.000060 | 0.060 | - |
| 6.0 | 4.63 | 0.000063 | 0.068 | - |
| 7.0 | 4.56 | 0.000080 | 0.080 | - |
| 8.0 | 4.52 | 0.000090 | 0.090 | 1.12×10^{-4} |
| 30.0 | 4.16 | 0.00040 | 0.40 | 1.34×10^{-4} |
| 60.0 | 3.94 | 0.00082 | 0.82 | 1.37×10^{-4} |
| 90.0 | 3.82 | 0.0016 | 1.6 | 1.79×10^{-4} |
| 120.0 | 3.74 | 0.0023 | 2.3 | 1.94×10^{-4} |
| 150.0 | 3.68 | 0.0029 | 2.9 | 1.96×10^{-4} |
| 180.0 | 3.64 | 0.0033 | 3.3 | 1.56×10^{-4} |
| 240.0 | 3.59 | 0.0045 | 4.5 | 1.54×10^{-4} |
| 360.0 | 3.49 | 0.0055 | 5.5 | 1.57×10^{-4} |
| 420.0 | 3.45 | 0.0064 | 6.4 | 1.58×10^{-4} |
| 480.0 | 3.41 | 0.0075 | 7.5 | 1.62×10^{-4} |

The average value of k for all times greater or equal to 30 hours is 1.66×10^{-4} hrs. $^{-1}$.

Experimental.

All experiments were carried out in a thermostatically controlled laboratory at 18 C°. The surface tension measurements were carried out with a Cambridge Du Nouy Tensionometer. A Marconi Electric pH-Meter was used for the pH measurements.

For the quick preparation of pure diacetimide in poor yield crude diacetimide distilled from the reaction mixture (2) is recrystallized 3 times from 12 - 15 parts of petroleum ether (60-80 C° to give a melting point of 80.0 - 80.5° (corr.) in a yield of 30 - 40%

The mother liquors or crude material may be recrystallized systematically to give good yields and melting points. The amount of solvent varies with the acetamide content of the crude preparation. In the average case the crude material is extracted by simmering 3 times with petroleum ether (12 cc. per 1 g.) for about 10 minutes and decanting from the undissolved molten acetamide layer. The extracts are allowed to crystallise separately. The melting points are taken and fractions with similar melting points are jointed for further recrystallization from petroleum ether. The fraction with the lowest melting point is combined with the residues obtained on evaporating the mother liquor. Further recrystallizations are carried out with decreasing amounts of solvent. The progress of purification is checked with the help of Figure 1. After 3-4 recrystallizations it is possible to obtain 70 - 80% of the crude material as pure diacetimide (mp. 79.5 - 80.5°, corr.) in addition to a fraction of pure acetamide. About 10 - 15% of the crude product remains in the mother liquor as diacetimide of 60 - 80% purity and may be kept for purification as stocks accumulate in further experiments.

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with references to earlier work.

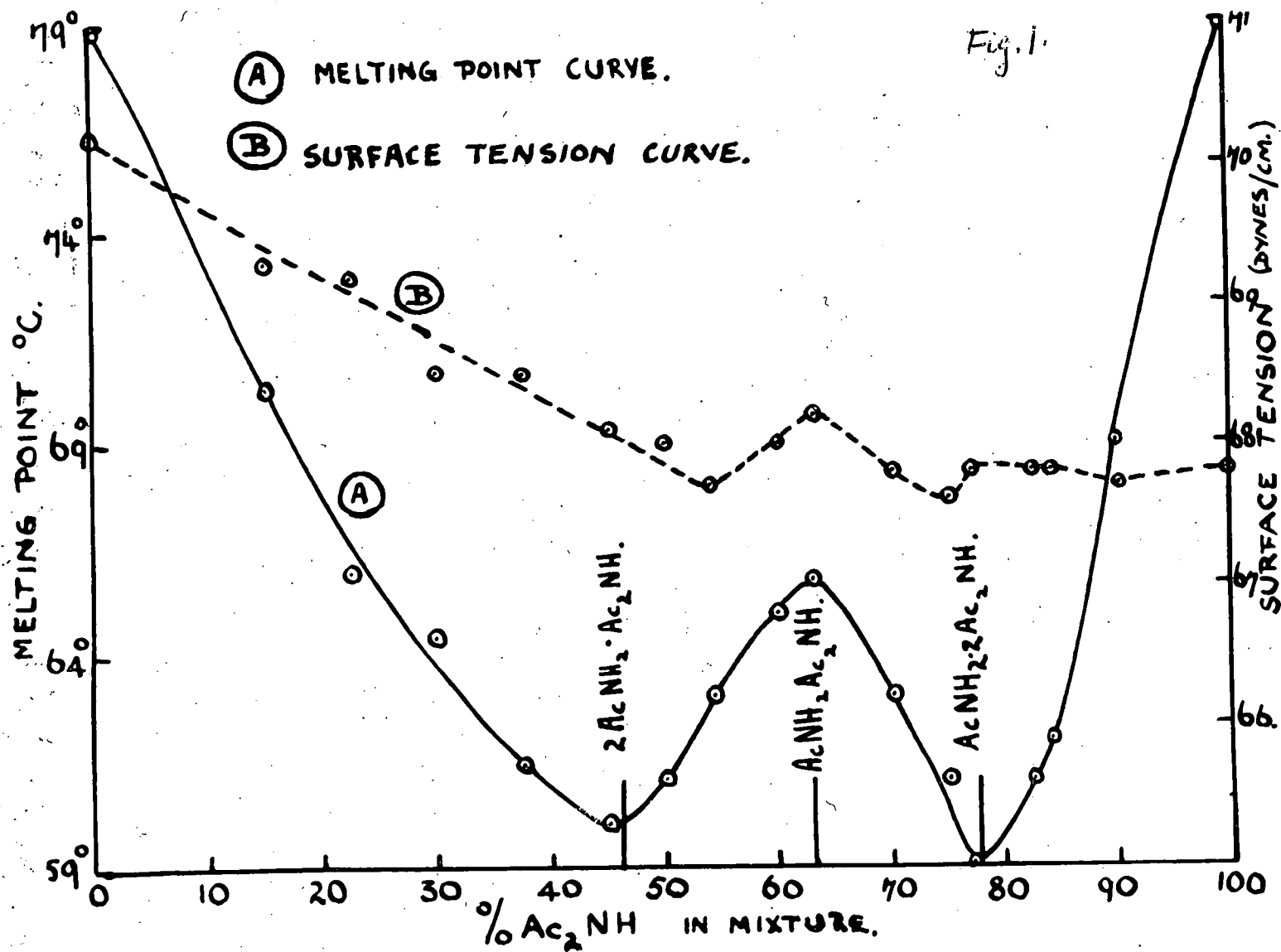


Fig. 2.

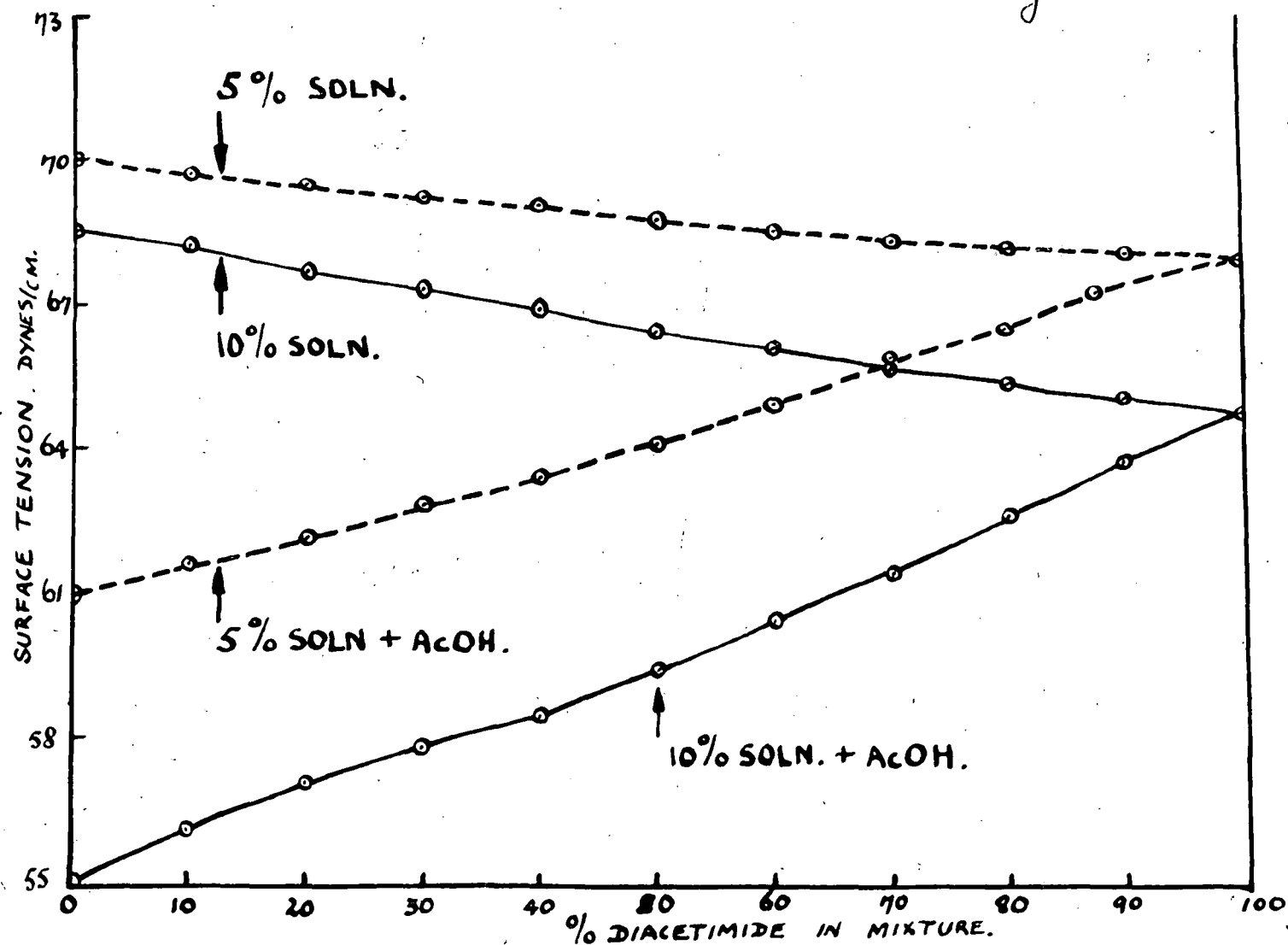


Fig. 3.

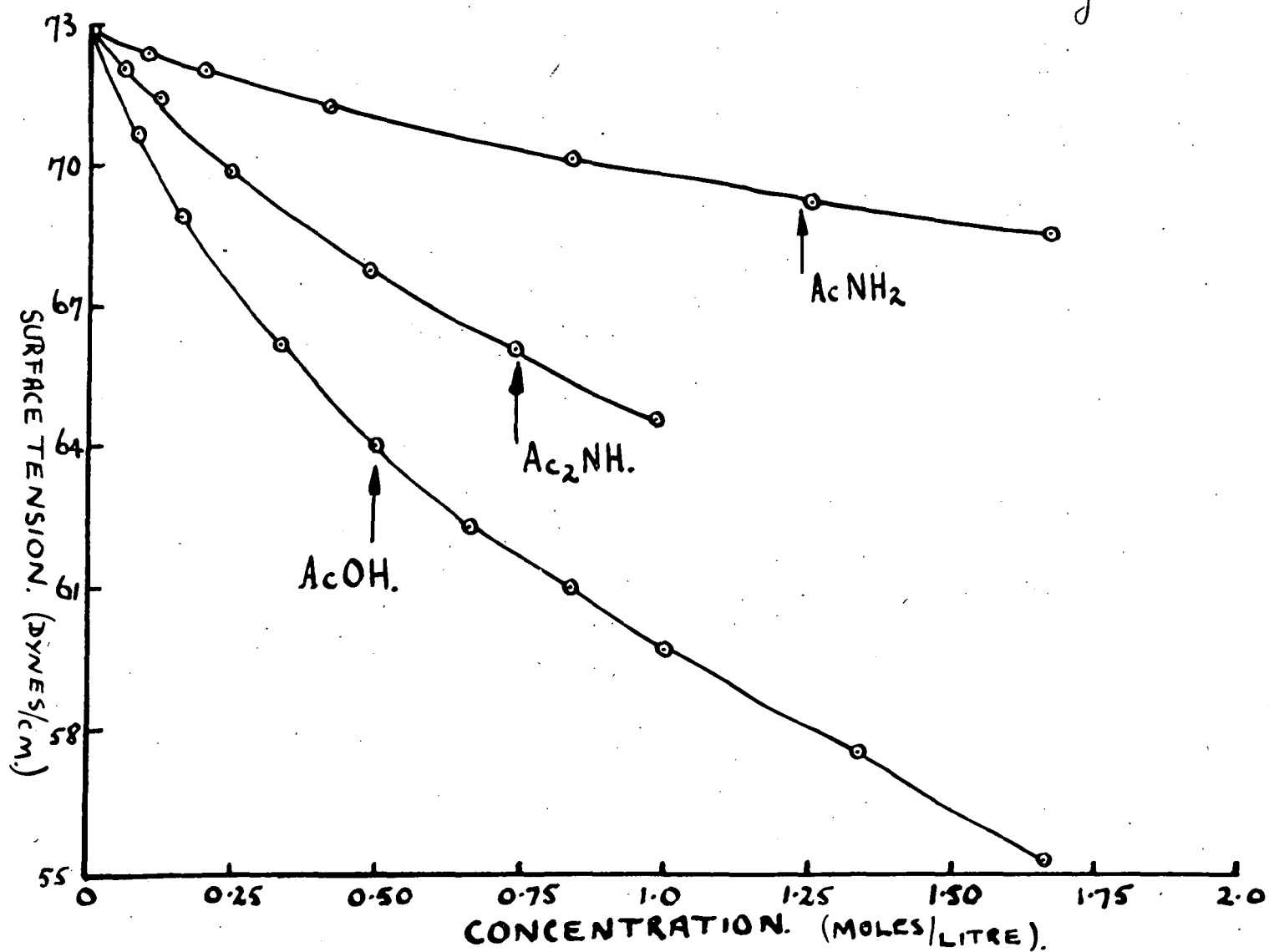


Fig. 4.

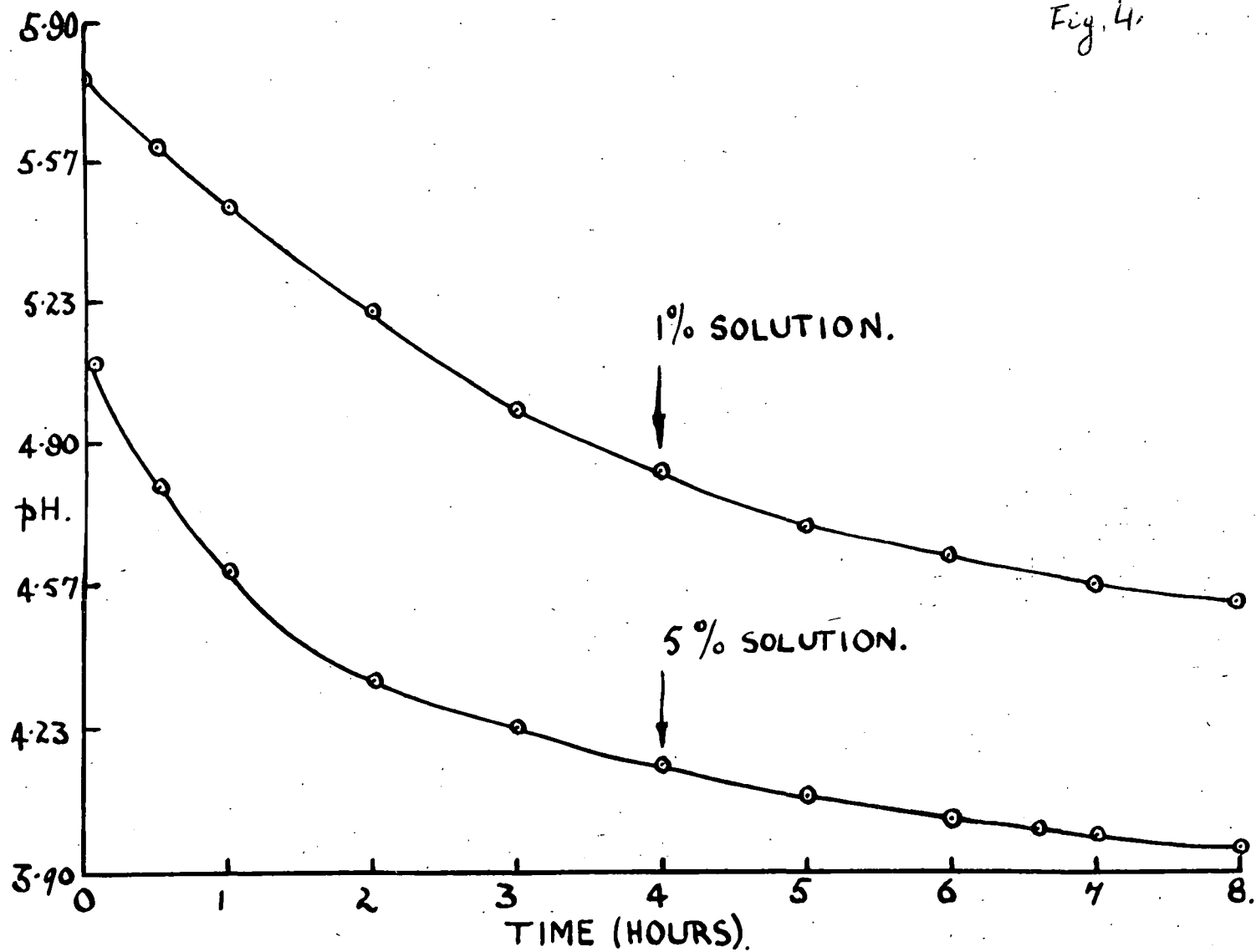
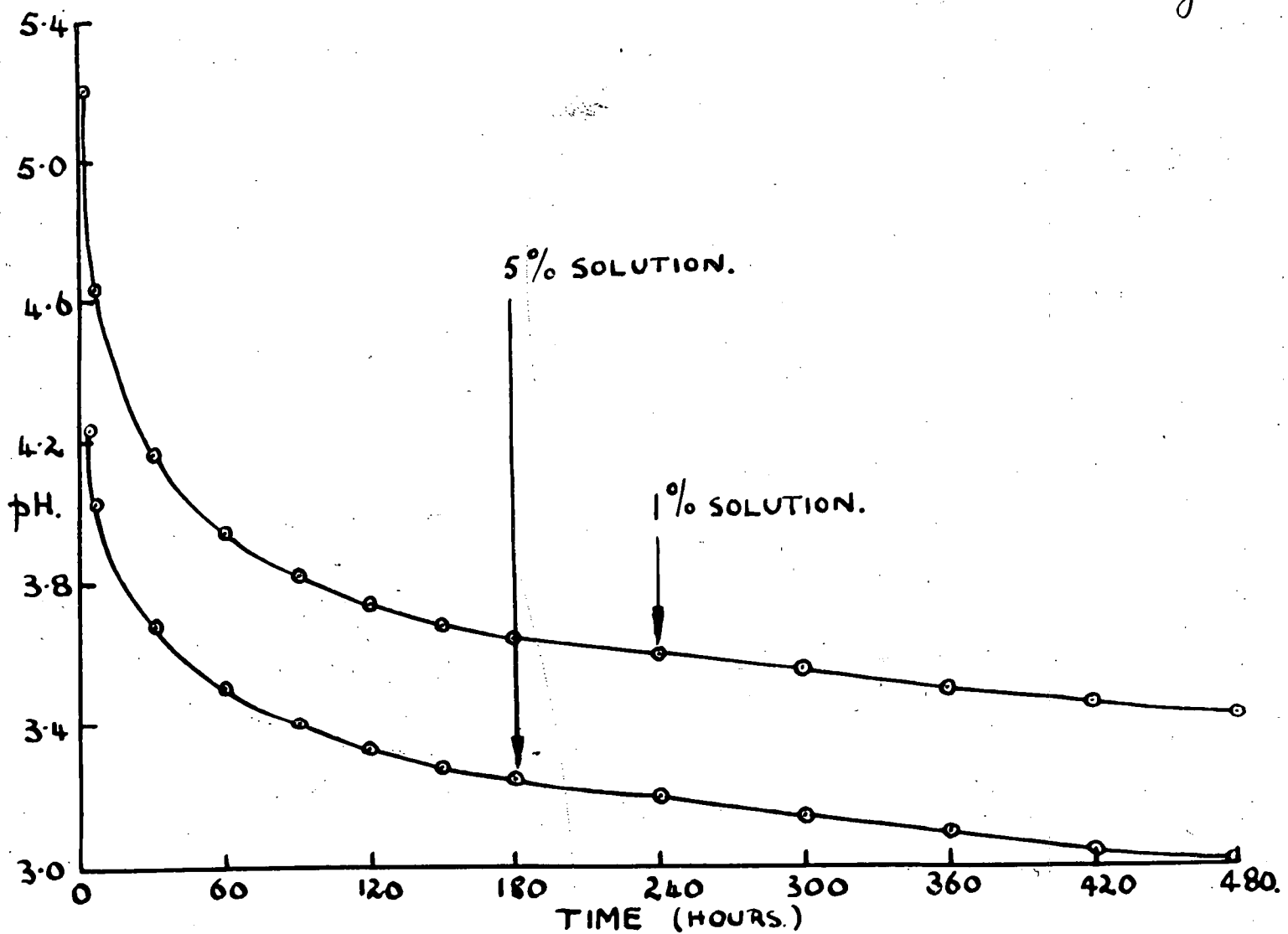


Fig. 5.



AMIDES VII. The Use of Diacylimines in the Perkin Reaction.

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Summary: The base catalysed reaction between diacylimines and aromatic aldehydes or furfural affords good yields of the expected unsaturated amides.

E.g. 3-(2-furyl)-2-methyl-2-propenoic amide, may be prepared by this method with a yield of 71%. Chloral and 3-(2-furyl)-2-propenal give poor yields of the expected unsaturated amides but these yields are higher than those obtained through the normal Perkin reaction. The mechanism of the Perkin reaction is discussed in the light of new experimental evidence.

It has been reported that diacetimide ($\text{Ac}_2\text{NH} = \text{I}$), reacts with benzaldehyde in the presence of sodium or potassium acetate to give cinnamamide as the main product and benzylidene bisamides as byproducts (1). The reaction has been extended to the use of other, symmetrical and mixed, diacylimines with benzaldehyde, some substituted benzaldehydes, furfural (FCHO), furyl acrolein (FCH:CHCHO) and chloral. The present paper is restricted to the discussion of that part of these experiments which deals with the Perkin reaction. A second publication will deal with the chemistry of bisamides some of which had to be prepared by methods other than the Perkin reaction. Reference will be made to the formation of bisamides in the present paper but preparative details and constitutional proofs will be reserved for the following publication in this series.

The general technique of the modified Perkin reaction was based on earlier work. Confirming earlier findings, it has been found that bisamide formation does not occur at lower reaction temperatures (125-150°); that the best yields of bisamides are obtained at reaction temperatures of about 180° in the presence of potassium acetate and that reaction temperatures of about 220° in the presence of sodium acetate give the best yields of amides with very low yields of bisamides. These observations refer to benzaldehyde and its derivatives. The use of furfural in the modified Perkin reaction requires lower temperatures to avoid resin formation. Bisamides of furfural do not appear to form under any of the investigated conditions. Under the optimum conditions of amide formation the amide/bisamide ratio is 18-19 or occasionally much higher in the benzaldehyde series. Under the optimum conditions favouring the formation of benzylidene bisamides the amide/bisamide ratios are only a little higher than 1. The preparation of 5-(2-furyl)-2-4-pentadienoic acid ($\text{FCH:CH=CH:CHCOOH} = \text{II}$) by the normal Perkin reaction (2) gives a yield of 6% only whereas the modified Perkin reaction affords the corresponding amide (not isolated) which after acid hydrolysis gives variable (9-20%) yields of the acid. The expected bisamides are not formed. Chloral in the normal Perkin reaction gives a 1% yield of 4,4,4-trichloro-3-hydroxy-butanoic acid whereas the modified Perkin reaction gives 5% of the corresponding unsaturated amide, $\text{Cl}_3\text{C.CH:CH.CONH}_2 = \text{III}$, presumably in the trans-form. Cold alkaline hydrolysis affords fumaric acid from either product. The latter experiments are of no practical value except for the indication that the modified Perkin reaction may be preferable in cases

where the normal Perkin reaction is ineffective.

According to our own experiments, partly substantiated by other evidence (3), the Knoevenagel, Doebner, Claisen and Reformatsky methods do not yield II from furyl acrolein. Further work on II and related compounds will be reported in another connection. In agreement with earlier observations, the formation of acids corresponding to the aldehyde or the expected amide is negligible in the benzaldehyde series: only a few milligrams of such acids can be isolated from m/10 experiments. In the case of furfural however, appreciable amounts of furoic acid are formed in addition to much resinous material which seems to take the place of bisamides.

In addition to I the following diacylimines have been used in this work; acetyl propionamide, $\text{AcNHCOEt} = \text{IV}$; dipropionimide, $(\text{EtCO})_2\text{NH} = \text{V}$; acetyl benzamide, $\text{AcNHCOPh} = \text{VI}$. The experimental conditions, products and yields are listed in Table 1. The amides listed are the following; cinnamamide, $\text{PhCH:CHCONH}_2 = \text{VII}$; α -methylcinnamamide, $\text{PhCH:CHMeCONH}_2 = \text{VIII}$; 3-(2-furyl)-2-propenoic amide, $\text{FCH:CHCONH}_2 = \text{IX}$; 3-(2-furyl)-2-methyl-2-propenoic amide, $\text{FCH:CHMeCONH}_2 = \text{X}$ and III. II obtained by the modified Perkin reaction from furyl acrolein and IX with X as obtained from FCHO and IV are listed under the amides although these have been isolated as acids only in these experiments. In the case of ring-substituted products the position and nature of the substituents are shown in brackets after the formulae of the parent compounds. Double rows of products in experiments with IV refer to the isolation of two major products. In the column headed "Acids" yields of corresponding acids obtained from the normal Perkin

reaction are shown
obtained from the
references in the
nearest integers

| Aldehydes | RCONHCOR' | |
|---------------------------|-----------|----|
| PhCHO | I | Ac |
| PhCHO | I | Ac |
| PhCHO | I | Ac |
| PhCHO | IV | Ac |
| PhCHO | IV | Ac |
| PhCHO | V | Ac |
| PhCHO | V | Ac |
| PhCHO | V | Ac |
| PhCHO | VI | Ac |
| PhCHO | VI | Ac |
| PhCHO(2-NO ₂) | I | Ac |
| PhCHO(3-NO ₂) | I | Ac |
| PhCHO(4-NO ₂) | I | Ac |
| PhCHO(2-Cl) | I | Ac |
| FCHO | I | Ac |
| FCHO | I | Ac |
| FCHO | IV | Ac |
| FCHO | IV | Ac |
| FCHO | V | Ac |
| FCHO | V | Ac |
| FCHO | VI | Ac |
| FCHO | VI | Ac |
| FCH:CHCHO | I | Ac |
| Chloral | I | Ac |
| Chloral | I | Ac |

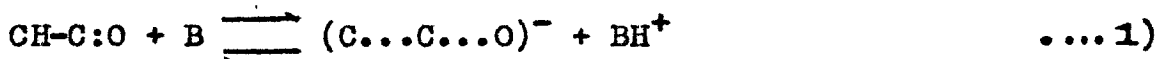
reaction are shown for comparison; some of these data have been obtained from the literature and are shown with the appropriate references in such cases. Yields have been rounded off to the nearest integers. Temperatures refer to those of the oil bath.

TABLE 1.

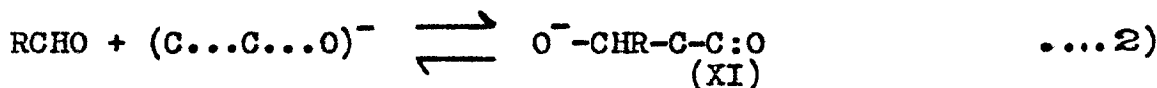
| Aldehydes | RCONHCOR' | Base | Temp. C° | Time Hrs. | Amides Products | Yield % | Acids yield |
|---------------------------|-----------|-------|----------|-----------|-------------------------|---------|-------------|
| PhCHO | I | AcONa | 220 | 8 | VII | 81 | 48-52(4,5) |
| PhCHO | I | AcOK | 180 | 5 | VII | 38 | 60-72(6,7) |
| PhCHO | I | AcONa | 150 | 14 | VII | 33 | |
| PhCHO | IV | AcONa | 220 | 8 | VII | 46 | |
| | | | | | VIII | 37 | |
| PhCHO | IV | AcOK | 180 | 5 | VII | 22 | |
| | | | | | VIII | 18 | |
| PhCHO | V | AcONa | 220 | 8 | VIII | 74 | 60-75(8) |
| PhCHO | V | AcONa | 140-150 | 20 | VIII | 89 | |
| PhCHO | V | AcOK | 180 | 5 | VIII | 40 | |
| PhCHO | VI | AcONa | 220 | 8 | VII | 88 | |
| PhCHO | VI | AcOK | 180 | 5 | VII | 39 | |
| PhCHO(2-NO ₂) | I | AcONa | 220 | 8 | VII(2-NO ₂) | 90 | 75(5-9) |
| PhCHO(3-NO ₂) | I | AcONa | 220 | 8 | VII(3-NO ₂) | 89 | 75(5) |
| PhCHO(4-NO ₂) | I | AcONa | 220 | 8 | VII(4-NO ₂) | 91 | 82-90(5,11) |
| PhCHO(2-Cl) | I | AcONa | 220 | 8 | VII(2-Cl) | 85 | 66-71(4,5) |
| FCHO | I | AcONa | 180 | 5 | IX | 74 | 65-70(10) |
| FCHO | I | AcOK | 140 | 3 | IX | 60 | |
| FCHO | IV | AcONa | 180 | 5 | IX (acid) | 40 | |
| | | | | | X (acid) | 32 | |
| FCHO | IV | AcOK | 140 | 3 | IX (acid) | 31 | |
| | | | | | X (acid) | 25 | |
| FCHO | V | AcONa | 180 | 5 | X | 71 | |
| FCHO | V | AcOK | 140 | 3 | X | 53 | |
| FCHO | VI | AcONa | 180 | 5 | IX | 83 | |
| FCHO | VI | AcOK | 140 | 3 | IX | 61 | |
| FCH:CHCHO | I | AcONa | 160 | 5 | II (acid) | 9-20 | 6 |
| Chloral | I | AcONa | 220 | 8 | III | 5 | 1 |
| Chloral | I | AcOK | 180 | 5 | III | 5 | |

The yields of pure amides from the modified Perkin reaction are somewhat higher than the yields of corresponding acids from the normal Perkin reaction. The conversion of acids to amides does not occur with theoretical yields, hence the modified Perkin reaction may be of some economic value in the direct preparation of amides in certain cases. The trends of yields in the acid and amide series in relation to the chemical types of the reactants follow identical courses and indicate that the mechanisms of the normal and modified Perkin reactions are identical or very similar. The criticism of some early theories of the Perkin reaction (1, 11) need not be repeated and we shall proceed to the analysis of the present results on the basis of the mechanism proposed by Broslow and Hauser (12).

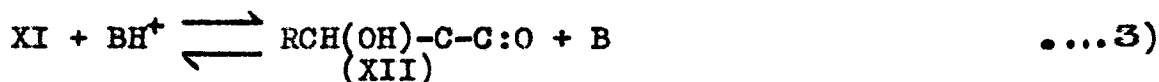
The first step is the enolization of the enolizable reactant (anhydride or diacylimine) by B, a basic catalyst:



The dotted lines in this and other equations indicate the existence of resonance ideals, C:C-O^- and $\text{C}^- \text{C:O}$ in this case. For the sake of greater clarity the essential skeleton only of the reactants is shown. The enolate anion reacts with the aldehyde:



Reaction between the conjugate acid of B and XI regenerates the base



and XII may undergo stabilization by further reactions which will be considered later. In the case of anhydrides the

enolizable reactant is not enolized in the absence of suitable catalysts, or small amounts of enol which may be present cannot change into the enolate anion. For such reasons the uncatalyzed normal Perkin reaction does not occur to any appreciable extent. The position is different in the case of diacylimines, for which it has been shown (13) that enolic forms are present in appreciable amounts even in the absence of catalysts. Furthermore the postulated enolic form $RC:C(OH)-N=C(OH)-R'$ is basic as shown by the existence of stable aluminium chloride complexes (14). Thus the formation of enolate anions is possible even in the absence of catalysts, and the modified Perkin reaction may proceed without catalysts (1).

Spectrographic evidence indicates that the diacylimines containing a propionyl rest are more enolized than those containing acetyl or chloroacetyl rests only. In addition to the increased extinction coefficients for diacylimines containing the propionyl radical against those containing acetyl or chloroacetyl radicals a red-shift is observed for the former. This indicates resonance stabilization of higher order, possibly through hyperconjugation. These two effects oppose each other with the result that the yields of amides from I, IV. and V. in either the benzene or furfural series are not significantly different. Both in the normal and the modified Perkin reaction dipropionyl derivatives give the best yields if the reaction temperature is decreased and the time of reaction increased. This may be due to relative instability at higher temperatures or to steric effects. Resonance stabilization does not account for this effect since lower temperatures and increased reaction

times reduce the yields in the cases of acetic anhydride and diacetimide, the resonance energies of which are not expected to be significantly lower than those of the corresponding propionyl compounds. Reactions with IV indicate that the increased enolization effected by replacing one acetyl group of diacetimide by the propionyl group affects the remaining acetyl group more strongly than the new propionyl group, since VII predominates over VIII and IX over X. The combined yields in these cases are significantly lower than those from experiments with VI. This is best explained by considering that the modified Perkin reaction VI may occur in one way only and will have a greater probability factor than reactions with IV. The reactive form of VI according to the Breslow-Hauser theory should be $\text{PhC}(\text{OH})\text{:N-C}(\text{OH})\text{:CH}_2$ (XIII), or rather its enolate. Spectrographic studies indicate the presence of some $\text{PhC}(\text{:O})\text{-N:C}(\text{OH})\text{CH}_3$ which is probably responsible for the hydrolytic and alcoholic reactions of VI (15, 16). It is clear, however, that the larger portion of VI is characterized by a spectral curve which is closely similar to that of benzamide. The latter must be assumed to exist mainly in the imidol form (17) related to XIII.

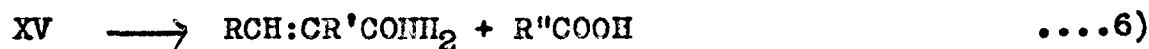
Addition of the enolate anions to aldehydes will be enhanced by substituents on the latter if these substituents tend to attract electrons or counteract the "flow" of electrons towards the carbonyl carbon atom thus increasing the polarity of the C:O bond. The effect of ortho, para or meta nitro substituents is readily understood. As regards o-chlorobenzaldehyde, it should be remembered that the mesomeric and inductive effects of halogen on the benzene nucleus are opposite and almost evenly

balanced and that chelation would promote the polarization of the C:O bond by an alternative mechanism. The lack of reactivity of furyl acrolein both in the normal and modified Perkin reaction cannot be explained at present. The well-known lack of reactivity of unsubstituted aliphatic aldehydes and of ketones is explained by the Robinson-negative inductive effect of the groups attached to the C:O group in all these cases, although better results are obtained if strong organic bases are used as catalysts (18). This argument breaks down in the case of chloral which, however, may present a case of a small, almost spherical molecule shielded by the strong negative fields of the chlorine atoms and the oxygen which prevent the effective approach of the enolate anion.

The existing information is not sufficient to establish the mechanism by which XII is converted into the acid or amide which constitute the major products of the normal or the modified Perkin reactions. Two possible mechanisms are the following:-



with subsequent hydrolysis of XIV or



In either case the final products are identical. The mechanism illustrated by equation 4 is unlikely, in view of the facts that at the reaction temperatures commonly employed most of the water would be removed and that the hydrolysis of diacylimines occurs more slowly than that of the anhydrides. Thus the higher yields of the modified as against the normal Perkin Reaction would be difficult to explain. Also we never succeeded

in isolating diacylimines of the type XIV. The argument that higher diacylimines of this type are unstable at the reaction temperatures used in this work is not valid since such instability is due to dissociation into the corresponding acids and nitriles. The latter might be converted into the required amides but the liberated acid could be isolated as such from the reaction product. When R" is the phenyl group, hydrolysis of XIV affords benzamide and the "aliphatic" acyl radical is changed into the acid. Similarly pyrolysis of XIV with R" = Ph would be expected to yield benzonitrile and benzamide in addition to cinnamic or furyl acrylic acid from experiments with VI. This is not the case and it is more plausible to assume that the stabilization of XIIa occurs mainly or exclusively through the mechanism represented by equations 5 and 6. There is some evidence for this mechanism from studies on the normal Perkin reaction (19). Evidence of a different kind from modified Perkin reactions is being completed and will be reported shortly. To complete the discussion, it should be pointed that an alternative way of internal acylation



may be excluded on two grounds. The formation of a β -lactone is unlikely on steric grounds. The normal Perkin reaction cannot settle this question since XVI would hydrolyse very readily to the experimentally found product. The modified Perkin reaction, however, excludes this possibility definitely since the acids corresponding to XVI are isolated in traces only and no benzamide is formed in experiments with VI.

EXPERIMENTAL PART

The isolation and purification of VII from experiments which do not afford other amides has been described previously (1). The effect of catalysts, temperature and time on yields is shown in Table 1. This section is restricted therefore to the description of representative experiments to illustrate the methods of isolating amides other than VII and separating mixtures of amides from experiments with IV. All amides reported in this section were hydrolysed to the corresponding acids which were identified by melting points and acid equivalents. The latter figures only are reported in this section.

1) PhCHO (10.6 g.), IV (18.4 g.) and freshly fused AcONa (5.4 g.) were heated under reflux at 220° in an oil bath for 8 hours. The mixture was poured into ice water containing about 1% sodium carbonate. The dark oil which separated was extracted with ether (100 cc.). Removal of the ether left an oily residue which solidified on washing with two 10 cc. portions of ether. The solid was recrystallized from chloroform and was identified as VIII (2.23 g.). Evaporation of the mixed ether and chloroform solutions gave an oil which was extracted by 20 cc. ether. Recrystallization of the resulting solid residue from chloroform gave more VIII. Combined yield of VIII. 5.94 g., 36.9%; mp. $127-8^{\circ}$; acid equivalent found $163^{\pm} 2$, calculated 162. The ether solution was worked up for VII by the usual method and gave 6.73 g., 45.8%; mp. $146-7^{\circ}$.

2) PhCHO (10.6 g.), V (20.6 g.) and freshly fused AcONa (5.4 g.) were reacted as before. The residue from the iced sodium carbonate solution was filtered and extracted with

ether (250 cc.). Since VIII is not very soluble in ether, the residue was extracted with cold alcohol (30 cc.) which removed the last traces of VIII without dissolving bisamides. The ether extract was treated with a saturated solution of sodium bisulphite for 24 hours, separated, washed with water and dried with anhydrous potassium carbonate. After removing the solvent the residue was recrystallized from hot water (7.3 g. VIII). The alcoholic extract was dried and recrystallized from hot water (4.55 g. VIII). Combined yield of VIII 11.85 g., 73.6%; mp. 127-8°; acid equivalent found $162^{\pm} 1$, calculated 162.

3) 2-Nitrobenzaldehyde (1.51 g.), I (1.62 g.) and AcONa (0.54 g.) were reacted as before. The ether extract of the residue from iced sodium carbonate solution was treated with sodium bisulphite, washed with water, dried and freed from solvent. Two recrystallizations from aqueous ethanol gave 2-nitrocinnamamide 1.72 g., 89.6%; mp. 184-5° reported 185° (20); acid equivalent $191^{\pm} 2$, calculated 193.

4) Repetition of Experiment 3 with 3-nitrobenzaldehyde gave 3-nitrocinnamamide 1.70 g., 88.6%; mp. 194-6° reported 195-6 (21, 22); acid equivalent found $191^{\pm} 2$, calculated 193.

5) Repetition of Experiment 3 with 4-nitrobenzaldehyde gave 4-nitrocinnamamide 1.81 g., mp. 213-215°. A small portion of this somewhat impure material has been recrystallized from 75 parts of aqueous ethanol and had then a mp. of 217°, reported 217° (21, 22). The crude yield was 94.3%. The somewhat lower yield in Table 1. refers to the yield of pure material.

6) 2-chlorobenzaldehyde (1.41 g.), I (1.62 g.) and AcONa (0.54 g.) were reacted and worked up as in the preceding three

experiments. Obtained 2-chlorocinnamamide 1.55 g., 85.2%; mp. 167-8°, reported 168° (21, 23); acid equivalent found 180 ± 2 , calculated 182.5.

7) FCHO (9.6 g.), I (16.2 g.) and AcONa (5.4 g.) were reacted at 180° for 5 hours. The residue from iced sodium carbonate solution was extracted with three 50 cc. lots of ether. The remainder of the residue was extracted with warm chloroform in which it was completely soluble. Acidification of the aqueous portion precipitated an acidic solid. On two recrystallizations from water this substance was identified as furoic acid 0.52 g., 4.4%; mp. 131-2°; acid equivalent found 114 ± 2 , calculated 112. The ether extract was worked up as in previous experiments. On two recrystallizations from aqueous alcohol IX was obtained. Yield 10.15 g., 74.2%; mp. 168-9°, reported 168-9 (21); acid equivalent found 136 ± 2 , calculated 138.

8) FCHO (4.8 g.), IV (9.2 g.) and AcONa (2.7 g.) were reacted at 180° for 5 hours and worked up as in previous experiment. The aqueous portion yielded furoic acid (2.03 g., 18.4%). The mixture of amides in the ether extract could not be separated. After hydrolysis with 10% NaOH and acidification with hydrochloric acid the organic acids were separated by systematic fractional crystallization from hot water in which 3-(2-furyl)-2-propenoic acid is more soluble than 3-(2-furyl)-2-methyl-2-propenoic acid. In this way we obtained 3-(2-furyl)-2-propenoic acid 2.78 g., 40.3%; mp. 141-2° and 3-(2-furyl)-2-methyl-2-propenoic acid 2.60 g., 32.1%; mp. 106-7°.

9) FCHO (9.6 g.), V (20.6 g.) and AcONa (5.4 g.) were reacted at 180° for 5 hours and worked up in the usual manner.

The aqueous portion yielded furoic acid 0.58 g., 5.2%. After the usual treatment of the ether extract two recrystallizations from aqueous alcohol gave X 10.75 g., 71.2%; mp. 135-6°. Hydrolysis with 10% NaOH and careful acidification with dilute hydrochloric acid gave 3-(2-furyl)-2-methyl-2-propenoic acid in a yield of 97%. Acid equivalent found 150 ± 2 , calculated 152; nitrogen found 9.19%, calculated 9.27%.

10) FCHO (1.92 g.), VI (5.21 g.) and AcONa (1.08 g.) were reacted at 180° for 5 hours and worked up in the usual manner. Systematic fractional crystallization of the combined organic acids from the aqueous portion yielded furoic acid 0.10 g. and benzoic acid 1.98 g. The ether solution was worked up as in Experiment 6) and yielded IX 2.28 g., 83.2%; mp. 168-9°.

11) Furyl acrolein (12.2 g.), acetic anhydride (25 g.) and AcONa (12.5 g.) were refluxed for 5 hours in an oil bath kept at 180°. The product was poured into 500 cc. 5% sodium carbonate. After extracting with ether and filtering from a small amount of resinous material the aqueous solution was acidified and extracted with ether. The ether extract was recrystallized twice from hot water and afforded 1.05 g., 6%, II of mp. 154°. The experiment was repeated by substituting I for acetic anhydride. The product was poured into 200 cc. 5% sodium hydroxide solution, extracted with ether and filtered and refluxed for 45 minutes. After acidification with a slight excess of hydrochloric acid, extraction with ether, and two recrystallizations of the ether extract from hot water II, mp. 153-4°, was obtained in yields varying between 1.5 and 3.3 g., 9-20%.

12) Chloral hydrate (8.3 g.), acetic anhydride (10.2 g.) and AcONa (16.4 g.) were reacted at 220° for 8 hours. The ether extract of the residue from iced sodium carbonate solution was discarded. The ether extract of the acidified aqueous solution contained 4,4,4-trichloro-3-hydroxy-butanoic acid. On recrystallizing from water 0.09 g., 1% was obtained; mp. 118-9°, reported 118-9° (24). Hydrolysis in the cold with alcoholic 10% KOH solution gave fumaric acid.

13) Chloral hydrate (8.3 g.), I (10.1 g.) and AcONa (16.4 g.) were reacted at 220° for 8 hours. The product was poured into ice water in which it was completely soluble. The ether extract of the aqueous solutions was transferred to petroleum ether (60-80°). On chilling long white needles separated. Two recrystallizations from a mixture of benzene and petroleum ether (4.1) yielded III 0.47 g., 5%; mp. 82-3°, reported 83° (25). Hydrolysis with 10% alcoholic KOH yielded fumaric acid.

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AMIDES VIII. Benzylidene and Furylidene Bisamides.

by

J.B. Polya and T.M. Spotswood

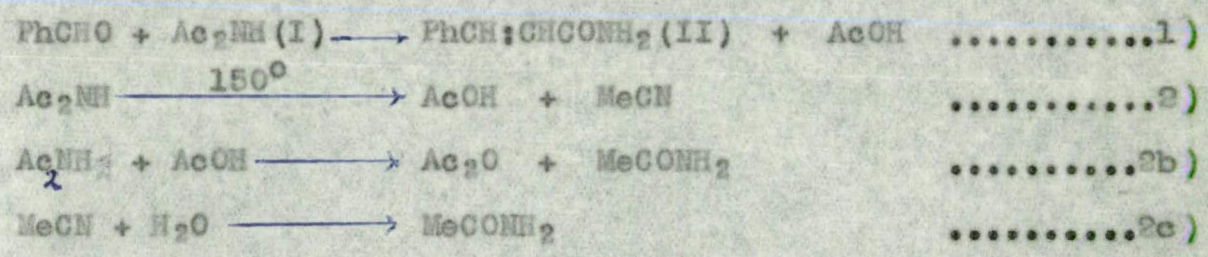
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Summary: The preparation of bisamides of the type $RCH(NHCOR')(NHCOR'')$ with R = phenyl or 2-furyl, R' and R'' = Me, Et, Ph, $PhCH:CH$ or $PhCH:OMe$, by the modified Perkin reaction or direct condensation of aldehydes and amides is described. Theoretical aspects of these preparations are discussed.

Symmetrical aliphatic and aromatic bisamides of the type $RCH(NHCOR')_2$ may be prepared by heating aldehydes with amides in the correct molecular proportions. This method has been investigated extensively by Pandya and his co-workers (1-16) who have also reviewed other methods of less general applicability. A more recent method of preparation for bisamides employs the reaction between benzaldehyde and diacylimines in the presence of potassium acetate at 180° which affords unsymmetrical bisamides, $PhCH(NHCOR')(NHCOR'')$ in good yields in addition to traces of symmetrical bisamides (17,18). Such unsymmetrical bisamides have not been obtained by other workers although their direct preparation from aldehydes and suitable amides presents no difficulties.

The preparation of bisamides by the modified Perkin method is of limited application since aliphatic aldehydes and furfural do not appear to react satisfactorily under the investigated conditions. If the modified Perkin reaction is carried out at temperatures below $130-140^\circ$ no bisamides are formed although

temperatures of this order are sufficient to prepare bisamides by the direct condensation of aldehydes and amides. Lack of bisamide formation at lower temperatures is not due merely to the absence of amides since the modified Perkin reaction affords these in appreciable amounts even at 130-140°. Furthermore a direct reaction between amides and benzaldehyde would be expected to favour the formation of symmetrical bisamides.

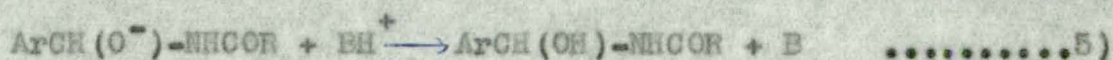
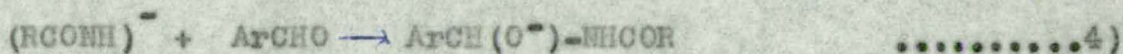


Reaction 1) occurs at 130-140° while the formation of acetamide from diacetimide in reactions 2a, 2b and 2c does not take place below 150° (19), and there is a high concentration of II in the reaction mixture at a time when acetamide begins to be formed through the pyrolysis of I. It is also known that while II displaces acetamide from benzylidene bisacetamide, $\text{PhCH}(\text{NHCOMe})_2 = \text{III}$, with the formation of benzylidene acetamide cinnamamide, $\text{PhCH}(\text{NHCOMe})(\text{NHCOCH:CHPh}) = \text{IV}$, displacement of cinnamamide from benzylidene biscinnamamide, $\text{PhCH}(\text{NHCOCH:CHPh})_2 = \text{V}$, by acetamide is very difficult or impossible (17). It appears then that the formation of bisamides in the modified Perkin reaction is governed by the relative rates of condensation of the $-\text{CH}_2-\text{CO}-$ and $-\text{NH}-\text{CO}-$ groups respectively. In the absence of basic catalysts or bases of intermediate strength (e.g. sodium acetate) the former condensation predominates while in the presence of stronger bases (e.g. potassium acetate) the two condensations may be of the same order of importance. A simple theory cannot be based on these considerations alone since

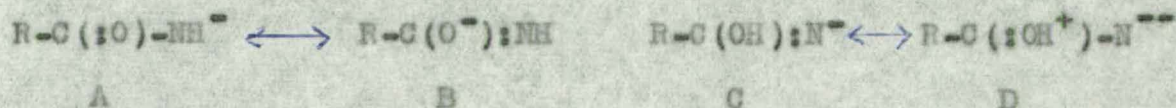
bases may catalyze or inhibit the formation of bisamides from aldehydes and amides. Pandya's work on symmetrical bisamides has established that pyridine inhibits the condensation of cinnamamide and heptaldehyde (14); the uncatalyzed condensations with piperonal and 2-nitropiperonal afford yields of 82-83% (7,8). The condensation of benzamide with m-tolylaldehyde is catalyzed by pyridine (11), condensations with piperonal, dihydrocinnamaldehyde and 5-chlorosalicylaldehyde are inhibited by pyridine (7, 13, 16), and in a large number of other condensations the catalytic or inhibitory effect of bases was either not tried or was ambiguous. The comparative difficulty of forming bisbenzamides was noted by Pandya and his collaborators and the same experience was derived from the present work. Condensations of acetamide were inhibited by basic catalysts in reactions with o-methoxybenzaldehyde (6), benzaldehyde (12), dihydrocinnamaldehyde (13), n-heptaldehyde (14), 6-bromopiperonal (15), and 3,5-dichlorosalicylaldehyde (16), and promoted in reactions with cinnamaldehyde (2), m-tolylaldehyde (11) and o-chlorobenzaldehyde (12). It should be mentioned, however, that the effects were not always very great and modifications of the experimental conditions do not permit a fully valid comparison of these data. More significant from a theoretical point of view are the observations of Pandya and co-workers that salicylaldehyde (1), p-hydroxybenzaldehyde (5) and m-hydroxybenzaldehyde (4) react with amides to give acylaldimines, ARCH:NCOR , only. Protection of the OH groups by methylation gives the normal bisamides (6). Introduction of chlorine into the nucleus of hydroxybenzaldehyde favours bisamide formation. Thus

3,5-dichlorosalicylaldehyde gives bisamides only while 5-chloro-salicylaldehyde gives bisamides with acetamide, propionamide and butyramide, and acylaldehydes with n-heptamide and benzene-sulphonamide, both types of products being obtained with benzamide (16). From this evidence and from the fact that nitro-groups in the nucleus favour bisamide formation it would appear that under the optimum conditions the CHO group should be electrophilic with the NH₂ group acting as donor of electrons. The difficulties encountered with benzamide are then explained by the mesomeric displacement of electrons of this substance from the amide group towards the aromatic nucleus. Furfural is less satisfactory than benzene for the preparation of bisamides either by the Perkin or the direct condensation method in accordance with these views.

The condensation mechanism may be regarded in the same light as the Perkin condensation (18,20). If B is a base



For the structure of the amide anion the following may be considered:



Resonance between the canonical forms A and B, the improbability of D and the formation of a C-H bond favour the stabilization of A in the transition complex. Lack of enolization in cinnamamide explains the usually better yields of bisamides when this compound is used instead of aliphatic amides like CH₂:CH(OH)NH₂.

The stabilization of $\text{ArCH}(\text{OH})\text{-NHCON}$ may occur in two different ways. With a strong displacement of electrons towards the $\text{CH}(\text{OH})$ group, ionization of the OH is facilitated and water is split out to give acylaldimines. The same effect is achieved by the use of acid catalysts. In the opposite case, a shift of electrons away from the $\text{CH}(\text{OH})$ group facilitates the approach of the amide anion and thus the anionic exchange.

The bisamides are insoluble in water ether and petroleum ether which permits separation from aldehydes, diacylimines and amides, the latter either from the reactants or from the products of the Perkin reaction. Unsymmetrical bisamides are considerably more soluble in alcohol or chloroform than the corresponding symmetrical bisamides. In the modified Perkin reaction the latter are formed in traces only. Their separation from such experiments has been omitted owing to difficulties of separating mixtures of symmetrical bisamides and owing to their ready accessibility through the direct condensation method. Bisamides are identified through hydrolysis with dilute mineral acids which decompose them into aldehydes and acids. When the isolation of the acids is not too involved they may be recovered in almost quantitative yields. The bisamides are stable to aqueous alkali. Attempts to resolve IV by chromatography over lactose failed. It should be remembered, however, that very high dilutions are required for this method, that the substance is not easy to dissolve in hydrocarbon solvents and that resolution need not be complete. In view of these difficulties it was not possible to carry out the experiments on a scale large enough to afford polarimetric concentrations permitting the observation of significant

The stablization of ArCH(OH)-NHCOAr may occur in two different ways. With a strong displacement of electrons towards the CH(OH) group, ionization of the OH is facilitated and water is split out to give acylaldimines. The same effect is achieved by the use of acid catalysts. In the opposite case, a shift of electrons away from the CH(OH) group facilitates the approach of the amide anion and thus the anionic exchange.

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rotations in various fractions.

Experimental Part

Results are tabulated in Table 1, 2, 3 and 4. Experiments listed in anyone of the Tables have been carried out under closely similar experimental conditions to permit a comparison of yields. For this reason only representative experimental procedures for each Table are being described in detail. In Table 1, yields and characteristic of some of Pandya's preparations are listed for comparison. Compounds listed in other Tables have not been prepared before with the exception of IV (17).

1) Symmetrical benzylidene bisamides.

Benzaldehyde (0.02 mol) and one of acetamide (VI), propionamide (VII), benzamide (VIII), cinnamamide (II) and α -methylcinnamamide (IX) (0.04 mol) were heated rapidly to 140° in an oil bath and allowed to react at this temperature for 4 hours. On completing the reaction the product was allowed to cool in a beaker. The mass was broken up, washed with water and ether to remove unreacted products. Recrystallization from ethanol (2-3rd times) gave the required products. Our results are compared with those of Bhatnagar and Pandya (12); columns head^{ed} "reported" refer to that work.

2) Unsymmetrical benzylidene bisamides (Table 2).

Benzaldehyde and two different amides (0.02 mol each) were reacted at 140° for 6 hours. After cooling, breaking up the mass and washing with water and ether the product was extracted with ethanol (50-70 cc.) at $50-60^{\circ}$. The residue of symmetrical bisamides was discarded. The filtrate was concentrated to half its original volume and filtered. Water was added dropwise until permanent

turbidity resulted. The solution was cleared by heating gently and allowed to stand in the refrigerator overnight. The resulting crystalline material was filtered and recrystallized twice from ethanol. The column headed "Acids" in Table 2, shows the experimental and theoretical recovery of the acid named in the same column.

3) Furylidene bisamides (Table 3.)

Furfural (0.02 mol) was reacted with an amide (0.04 mol) or two different amides (0.02 mol each) at 100-120° for 2 hours. The crude products were isolated as described before. The recrystallization of symmetrical bisamides from ethanol was found to be unsatisfactory and toluene (100-150 cc.) was used. Unsymmetrical bisamides were isolated by the method given under 2). Mixed amides VI, VIII and VII with VIII gave resinous products only.

4) Unsymmetrical bisamides from the Perkin reaction (Table 4.)

The ether insoluble fraction of the products of the previously described modified Perkin reaction (18) were worked up for unsymmetrical bisamides by the method given under 2). The diacylimines used were diacetimide (I), dipropionimide (X) and acetylbenzamide (XI). Acetyl propionamide did not afford any of the expected bisamides.

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TABLE 1.

 $\text{PhCH}(\text{NHCOR})_2$

| Amide | R | Yield% | | m.p. °C | | N% | | | MW | | Remarks |
|-------|--------------------------|--------|----------|---------|----------|-------|-------|----------|-------|-------|--|
| | | found | reported | found | reported | found | calc. | reported | found | calc. | |
| VI | Me | 49.1 | 55.0 | 244.5 | 245 | 13.54 | 13.59 | 13.84 | 220 | 206 | |
| VII | Et | 45.1 | 30.7 | 225 | 220 | 11.83 | 11.97 | 12.56 | 240 | 234 | |
| VIII | Ph | 27.3 | 63.6 | 217.8 | 217.5 | 8.42 | 8.48 | 8.73 | 340 | 330 | |
| II | $\text{PhCH}:\text{CH}$ | 62.0 | 80.0 | 249.50 | 238 | 7.34 | 7.33 | 7.26 | 390 | 382 | |
| IX | $\text{PhCH}:\text{CMe}$ | 48.1 | - | 201 | - | 6.81 | 6.83 | - | 430 | 410 | gives 76.1 $\text{PhCH}:\text{CMeCO}$ calc. 79.0 |

TABLE 2.

PhCH (NHCOR^I) (NHCOR^{II})

| Amides | R ^I | R ^{II} | Yield % | m.p. °C | N% | | M W | | Acid% | | structure |
|-----------|----------------|-----------------|---------|------------|-------|-------|-------|-------|-------|-------|---------------|
| | | | | | found | calc. | found | calc. | found | calc. | |
| VI, II | Me | PhCH:CH | 39.6 | 214-5 | 9.50 | 9.52 | 315 | 294 | 48.2 | 50.4 | PhCH:CHCOOH |
| VI, VII | Me | Et | 30.2 | 227-8 | 12.65 | 12.73 | 242 | 220 | | | |
| VI, VIII | Me | Ph | 42.7 | 206 | 10.41 | 10.45 | 280 | 268 | 43.2 | 45.5 | PhCOOH |
| VI, IX | Me | PhCH:CMc | 38.0 | 182-3 | 9.05 | 9.09 | 325 | 308 | 49.3 | 52.6 | PhCH:CMc:COOH |
| VII, VIII | Et | Ph | 38.5 | 189-90 | 9.87 | 9.93 | 307 | 282 | 52.3 | 57.5 | PhCOOH |
| VII, II | Et | PhCH:CH | 33.1 | 230-1 | 9.03 | 9.09 | 321 | 308 | 45.2 | 48.1 | PhCH:CHCOOH |
| VII, IX | Et | PhCH:CMc | 30.4 | 187-8 | 8.65 | 8.70 | 350 | 322 | 46.3 | 50.3 | PhCH:CMcCOOH |
| VIII, II | Ph | PhCH:CH | 29.7 | 205 | 7.80 | 7.86 | 372 | 356 | | | |
| VIII, IX | Ph | PhCH:CMc | 26.1 | 191-2 | 7.51 | 7.57 | 391 | 370 | | | |
| II, IX | PhCH:CH | PhCH:CMc | 25.0 | 206 | 7.01 | 7.07 | 423 | 396 | | | |

TABLE 3

FCH(NHCOR^I)(NHCOR^{II}), F = 2 furyl

| Amides | R ^I | R ^{II} | Yield | m.p. °C. | N% | | M W | | Acids % | | structure |
|--------|----------------|-----------------|-------|-------------|-------|-------|-------|-------|---------|-------|-----------|
| | | | | | found | calc. | found | calc. | found | calc. | |
| VI | Me | Me | 50.8 | 210 dec. | 14.21 | 14.29 | 210 | 196 | | | |
| VII | Et | Et | 46.2 | 216 dec. | 12.39 | 12.50 | 238 | 224 | | | |
| VIII | Ph | Ph | 40.0 | 128 | 8.71 | 8.75 | 332 | 320 | 74.1 | 76.25 | PhCOOH |
| VI VII | Me | Et | 24.8 | 204-5 | 12.96 | 13.33 | 241 | 224 | | | |
| VI II | Me | PhCH:CH | 26.0 | 211-12 | 9.71 | 9.86 | 330 | 305 | 61.3 | 64.6 | PhCH:CHCO |

TABLE 4.

Amides and $\text{PhCH}(\text{NHCOR}')(\text{NHCOR}'')$ from the modified Perkin reaction

| Diacylimine | Catalyst | Temp. °C. | Time, hours | Amide | | R' | Bisamide | | yield % |
|-------------|----------|-----------|-------------|-----------|---------|----|----------|---------|---------|
| | | | | structure | yield % | | R'' | yield % | |
| I | AcONa | 220 | 8 | II | 81.2 | Me | PhCH:CH | 4.4 | |
| I | AcOK | 180 | 5 | II | 37.5 | Me | PhCH:CH | 36.2 | |
| X | AcONa | 220 | 8 | IX | 73.6 | Et | PhCH:CMe | 3.9 | |
| X | AcOK | 180 | 5 | IX | 40.1 | Et | PhCH:CMe | 37.1 | |
| XI | AcONa | 220 | 8 | II | 88.1 | Me | PhCH:CH | trace | |
| XI | AcOK | 180 | 5 | II | 39.1 | Me | PhCH:CH | 32.1 | |

The Use of Acetamide in the Meiostagmin Reaction.

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The original meiostagmin reaction (IR) was based on the reduction of the surface tension of cancer serum after incubation with tumor extracts (1-4). The numerous variations and modifications of this method have been reviewed by Stern and Willheim (21). The IR shares the lack of specificity with other lability tests but, apart from pregnancy, only very few non-cancerous complaints are known to interfere, and these are readily diagnosed by clinical methods and auxiliary techniques of the IR (14, 23). Keller and Künzel (11) used Lecomte du Nouy's dynamic principle (13) to observe surface tension variations in serum - sodium oleate systems. Significant differences between cancer and normal sera were noted. The surface tension buffering power of sera against strong surface active reagents has been investigated in connection with the IR but buffering in the presence of reagents expected to raise the surface tension of cancer sera have not been investigated so far. The present work describes experiments of the latter kind. Keller and Künzel explained their results by a combination of soap and serum proteins. More recent work (5) supports such views and indicates that similar combinations may take place between amides and serum proteins. For reasons of simplicity the effects of acetamide, urea and diacetamide (15) were tested in our experiments. The first two are denaturing agents, the former being weaker than the latter (20). The denaturing effect of diacetamide is the same

as that of acetamide judging from the titration of various proteins in isotonic aqueous saline with absolute alcohol to nephelometric standards using Keller's method (10).

METHODS.

Cancer and normal sera were taken at random at the Royal Hobart Hospital, Launceston General Hospital and Sydney Hospital in 5 - 10 mls. lots under sterile conditions. Oxalated and citrated sera were found unsuitable. The samples were defibrinated at the sampling stations. Different methods of defibrination did not effect the results. Slightly hemolysed samples did not effect the results contrary to observations with an early form of the MR (19).

The effects of delays in testing are shown in Table 3. Centrifuging immediately after sampling gave better quantitative results. Sodium cyanide helps to counteract the effects of delay in testing but it was not used in the reported experiments. Sodium fluoride has no effect and copper sulphate an adverse effect.

The sera were diluted with 5% aqueous solutions of the amides. With lower concentrations the results became inaccurate and higher concentrations were avoided to minimize denaturing effects. The amide solutions were added to the sera up to 512 dilutions. The solutions were homogenized by gently tilting the stoppered test tubes ten times, pouring into standard watch glasses of 4 mls. capacity with a surface: volume ratio of 4.0 and allowing the solutions to come to equilibrium. The latter required a few minutes and 3 - 4 preliminary measurements. The surface tensions (γ) of the original and diluted sera were measured at 18°C in a

thermostatically controlled room with a Cambridge Du Nouy Tensionometer. The average of 3-6 measurements was taken as the correct value as soon as the measurements became constant within ± 0.1 dyne/cm. Slightly fermented sera have low surface tensions in the undiluted state ($\gamma' = 49-52$ dyne/cm) and were discarded.

RESULTS AND DISCUSSION

With urea ($\gamma' = 73.0$ dyne/cm in 5% solution) no significant effect could be observed. Since weaker denaturing agents like acetamide and diacetamide gave more pronounced effects, these must be due mainly to causes other than denaturation. No consistent results could be obtained with diacetamide ($\gamma' = 67.8$ dyne/cm in 5% solution) apart from a sharp initial drop of the surface tension at low dilutions (7).

With acetamide purified by distillation alone curves of different shapes could be obtained for cancer and normal sera but the findings could not be duplicated with accuracy. The effect is ascribed to the odorous impurity of acetamide which is absent when acetamide is repeatedly recrystallized from benzene and ethyl acetate, and washed with ether. Acetamide solutions keep unchanged for 4 hours but inaccurate results are obtained with solutions 24-30 hours old.

Using fresh 5% solutions of acetamide ($\gamma' = 70.1$ dyne/cm) the surface tension versus dilution curves rise to a maximum at 16-50 dilutions. At further dilutions transient cloudiness appears and the surface tension values fall to a minimum at 100 - 200 dilutions. This minimum value (γ'_{\min}) is lower than the surface tension of the undiluted serum (γ'_0) with cancer,

pregnancy and some other sera. Since the rise to higher surface tension values past the minimum may be slow or fast, it was attempted to characterise the general behaviour of the curves by a conventional measure termed "critical area" (CA). A positive CA is defined as the area enclosed by the curve and a straight line drawn through γ_0 parallel with the dilution axis. If this line is a tangent of the curve at its minimum the CA is reported as 0. Otherwise the CA is reported as negative (-). In sera exhibiting powerful buffering effects around the value of γ_{min} it may happen that 512 dilutions are not sufficient to circumscribe the positive CA. In a few cases of this kind the area was completed by a line drawn perpendicularly through the point corresponding to 512 dilutions on the abscissa. A positive CA may be evaluated by counting squares or by planimetry. Figures reported in Table 1, 2, 3 and 4 have been rounded off to the nearest ten with an average error of about 10 units. Examples of typical cancer (C 41S) and non-cancer (N 11) curves are shown in Figure 1.

The use of CA values has the statistical disadvantage of unsymmetrical representations but CA values are correlated with the symmetrical expression $C = \gamma_0 - \gamma_{\text{min}}$. Results on cancer sera are shown in Table 1, and other sera in Table 2. An analysis of CA values by anatomical sites indicates that great variations are not to be expected (Table 4.).

Cases C42, C43 and C44 show the effects of blood transfusion. The CA values in the latter two cases are not comparable with the rest of the data. In the normal series no clinical data were available on Cases N4 and N15. Radiologically treated

cases appear to be correlated with flat, elongated curves. It is reasonable to assume that the low CA of C37L is a consequence of successful radiological treatment but the same could not be said about C12, a case of carcinoma of the stomach treated with penicillin.

Excluding cases C43 and C44 in the cancer series and those of pregnancy, one obtains average CA values (rounded off to the nearest ten) of 220 for 41 cancer cases and 40 for 11 non-cancer cases with positive or zero CA values. Better percentages could be quoted by omitting post-operative or radiologically treated cases with low CA values. The average values of G (rounded off to the first decimal) are 2.5 for 4 pregnancy, 1.4 for 42 cancer and -0.8 for 27 non-cancer cases. Only one non-cancer and non-pregnancy case (4%) exceeds the average G value for cancer, and all cancer cases have G values above the non-cancer and non-pregnancy average. A histogram of the G values is shown in Figure 2. illustrates the difference between the results obtained in the cancer and non-cancer series (the latter excluding cases of pregnancy. The difference between the corrected standard deviations for G in the cancer and non-cancer series is 0.38 ± 0.19 . A better indication of the significance of the MR is obtained from the t-test. For the calculated 8.0 for t and 66 freedoms a probability percentage point of well below 0.1% is obtained. A variance test gives $F(25, 41) = 2.0$ corresponding to a probability percentage point of about 3. The significance of the CA values in the cancer and non-cancer series can be estimated by the χ^2 - test. Dividing the samples into cancer and non-cancer classes and classes with positive and negative (including zero) CA values, is obtained as 39.5 which, for 1 degree of freedom, corresponds to a probability percentage

point of well below 0.1%. Practically the same result is obtained if the χ^2 -test is applied to the G values with classes comprising positive and negative (the latter including zero) G values.

The effect of dilution with buffer solutions has been studied in the case of cancer, diabetes and normal sera. Figures 3. and 4. show the results of a typical set of experiments on sample C12 which may be regarded as intermediate between typical cancer and non-cancer sera. With borate and succinate buffers (6) the surface tension versus dilution curves are different from those obtained with acetamide solutions and approximate the curves obtained on diluting sera with water (Figure 3.). The variation of the surface tension with pH exhibits maxima between 7.3 and 7.8 (12.).

The surface tension versus dilution curves present a close analogy with the curves obtained for the system diluted serum - sodium cholate (22) which show a sharp surface tension minimum at 20 - 200 dilutions, approximately as in our experiments. This effect has been explained by the combination of serum proteins with sodium cholate which tends to displace lipids from the lipoproteins. The effect increases with sera from which the lipids have been removed. This, however, is difficult to apply to the MR since γ_0 values of cancer sera are not higher than those of normal sera in spite of the raised lipid content of the former, and the surface tension maximum observed by Teyssu and Blanquet with lipid-free sera and in our experiments is not explained. On the other hand Elkes and Finean in their work on the hemoglobin - sodium hexadecyl sulfate system noted the formation of soluble and insoluble complexes at significantly different, limited ratios of detergent to protein (8). Elkes and Finean suggest different mechanisms of combination

on the acid and alkaline sides of the isoelectric point. In the case of the amphoteric acetamide and approximately symmetrical behaviour is expected. The behaviour of the chemically simpler system acetamide-diacetamide (7) confirms the suggestion that the characteristic lowering of the surface tension in our IR is due to a combination of acetamide with serum proteins resulting in the attraction of protein molecules into the surface. According to modern views on the nature of acetamide (17,18) the combination is assumed to take place through hydrogen bonding. The finer mechanism of the process, in particular whether film penetration occurs (7), cannot be decided from the existing evidence. The maximum of the curves may represent the opposite process which is reversed when a certain stage of denaturation has been achieved. At dilutions beyond this point a cloudiness occurs. This is not due to the precipitation of globulins owing to dilution since globulin fractions of cancer sera dissolved in salt to the protein concentration of the original serum give a IR with acetamide which is almost identical with the IR of the original serum. Finally it should be noted that the IR with acetamide is closely related to modern techniques of tensiometric titrations (16, 24).

Summary

- 1) The surface tensions of cancer and non-cancer sera were determined in progressive dilutions with 5% acetamide.
- 2) The difference between the initial and minimum surface tension values was found to be positive in pregnancy and most of the cancer samples whereas it was negative in most of the other samples.

- 3) A conventional unit, the critical area, was defined and found positive in pregnant and most of the cancerous samples whereas it was negative or slightly positive only in other samples.
- 4) The modified LR is discussed from the point of view of the combination theory.

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TABLE 1.

| Number | Sex | Age | Diagnosis | Treatment | o | min | G | CA |
|--------|-----|-----|---|----------------|------|------|------|-----|
| C 1 | F | 60 | C., cervix of uterus | ... | 58.0 | 56.0 | 2.0 | 320 |
| C 2 | M | 54 | C., stomach | ... | 58.0 | 54.2 | 3.8 | 950 |
| C 3 | F | 44 | C., cervix of uterus | ... | 57.0 | 55.0 | 2.0 | 370 |
| C 4 | F | 62 | C., uterus | ... | 55.0 | 53.8 | 1.2 | 80 |
| C 5 | F | 35 | C., ovaries | morphia | 57.2 | 53.4 | 3.8 | 910 |
| C 6 | F | 75 | C., cervix of uterus | ... | 57.3 | 53.8 | 3.5 | 210 |
| C 7 | M | 61 | C., lower bowel | O(5d.) | 59.0 | 57.9 | 1.1 | 90 |
| C 8 | F | 58 | C., lower bowel | O(2w.) | 58.5 | 57.8 | 0.7 | 50 |
| C 9 | M | 66 | C., head of pancreas | O | 58.2 | 55.9 | 2.3 | 520 |
| C 10 | F | 62 | C., breast, metastases | O(1w.) | 57.0 | 55.3 | 1.7 | 370 |
| C 11 | M | 57 | C., stomach, metastases | morphia | 57.0 | 55.6 | 1.4 | 320 |
| C 12 | M | 62 | C., stomach | O penicillin | 57.5 | 57.1 | 0.4 | 30 |
| C 13S | F | 68 | C., breast | O | 56.0 | 55.2 | 0.8 | 60 |
| C 14S | F | 40 | C., cervix of uterus | ... | 58.9 | 57.3 | 1.6 | 210 |
| C 15S | M | 63 | Squasm. carc. ani | ... | 55.3 | 54.3 | 1.0 | 290 |
| C 16S | M | 51 | Carc. piriform Fossa | ... | 56.9 | 54.6 | 2.3 | 380 |
| C 17S | M | 59 | Squasm. carc., hypopharynx | ... | 56.2 | 55.7 | 0.5 | 30 |
| C 18L | F | 49 | Carc., cheek | X, O(7 m.) | 57.4 | 54.8 | 2.6 | 650 |
| C 19L | M | 45 | Astrocytoma pariet | X, P | 57.8 | 56.7 | 1.1 | 70 |
| C 20L | M | 49 | Carc., larynx | X | 58.9 | 57.9 | 1.0 | 270 |
| C 21L | F | 63 | Carc., breast | X, P | 58.0 | 55.3 | 2.7 | 490 |
| C 22L | F | 74 | Carc., tongue | X | 58.0 | 57.1 | 0.9 | 100 |
| C 23L | F | 47 | Astrocytoma pariet | X, P | 57.7 | 56.8 | 0.9 | 130 |
| C 24 | F | 59 | Endocervic carc., II. | ... | 59.5 | 57.8 | 1.7 | 190 |
| C 25S | M | 36 | Carc., bladder | O(11 m.) | 57.0 | 56.7 | 0.3 | 10 |
| C 26S | M | 62 | Squasm. carc., lip | ... | 58.4 | 57.4 | 1.0 | 80 |
| C 27L | M | 72 | Carc., jaw, 7 years | X | 57.8 | 56.7 | 1.1 | 210 |
| C 28L | F | 38 | Pituitary tumor | X | 57.4 | 56.6 | 0.8 | 110 |
| C 29L | F | 76 | Squasm. carc., temple | X | 56.5 | 54.8 | 1.7 | 280 |
| C 30L | F | 55 | Adenocarc., uterus | ... | 56.7 | 56.0 | 0.7 | 80 |
| C 31S | F | 68 | Carc., cervix, II. | ... | 57.8 | 57.2 | 0.6 | 50 |
| C 32S | F | 39 | Carc., cervix, II. | ... | 58.8 | 57.7 | 1.1 | 150 |
| C 33S | F | 54 | Scirr. carc., breast | O (4 m.) | 56.0 | 56.0 | 0.0 | 0 |
| C 34L | F | 71 | Carc., breast, 22 years | ... | 57.0 | 55.8 | 1.2 | 100 |
| C 35L | F | 38 | Epithelioma, nose | ... | 58.0 | 56.7 | 1.3 | 230 |
| C 36L | F | 66 | Carc., breast | ... | 57.1 | 55.9 | 1.2 | 260 |
| C 37L | M | 52 | Carc., neck and chin | X | 56.4 | 57.0 | -0.6 | - |
| C 38S | F | 35 | Carc., cervix, II. | radiother. | 57.0 | 56.0 | 1.0 | 190 |
| C 39S | M | 60 | Obstruction of oesophagus, snaplast.carc. | ... | 56.3 | 55.8 | 0.5 | 40 |
| C 40S | M | 52 | Malignant angioma | radiother. | 56.8 | 56.0 | 0.8 | 70 |
| C 41S | M | 60 | Carc., lip | ... | 57.2 | 55.3 | 1.9 | 230 |
| C 42 | F | 62 | C., breast | O B(4d.) | 56.8 | 55.0 | 1.8 | 230 |
| C 43 | M | 60 | C., pancreas | O(1w.), B(1d.) | 54.9 | 55.5 | -0.6 | - |
| C 44 | M | 48 | C., oesophagus | B(2 d.) | 53.0 | 56.2 | -3.2 | - |

L: samples from Launceston. S: samples from Sydney. unmarked samples from Hobart.

O: operated. B: transfusion of 2 pints of blood. d: days. w: weeks.

m: months of operation or transfusion before test. X: X-ray treatment.

PB: phenobarbitone.

TABLE 2.

| Number | Sex | Age | Diagnosis | o | min | G | CA |
|--------|-----|-----|---------------------|------|------|------|-----|
| N 1L | M | 65 | Gastr. hyperacidity | 57.2 | 57.1 | -0.1 | 0 |
| N 2 | M | 25 | ... | 55.0 | 56.3 | -1.3 | - |
| N 3 | F | 25 | ... | 55.2 | 55.5 | -0.3 | - |
| N 4 | F | 20 | ? | 57.8 | 56.8 | 1.0 | 80 |
| N 5 | M | 24 | ... | 58.0 | 57.5 | 0.5 | 30 |
| N 6 | M | 26 | ... | 58.0 | 58.9 | -0.9 | - |
| N 7 | M | 21 | ... | 58.6 | 59.0 | -0.4 | - |
| N 8 | M | 39 | ... | 52.0 | 54.7 | -2.7 | - |
| N 9 | M | 18 | ... | 57.0 | 56.5 | 0.5 | 20 |
| N 10 | M | 23 | ... | 56.5 | 57.1 | -0.6 | - |
| N 11 | M | 24 | ... | 56.1 | 58.0 | -1.9 | - |
| N 12 | M | 42 | Fract. tibia | 55.0 | 56.7 | -1.7 | - |
| N 13 | F | 31 | ... | 56.0 | 59.2 | -3.2 | - |
| N 14 | M | 30 | Traum. hydrocele | 57.8 | 57.8 | 0.0 | 0 |
| N 15 | F | 62 | Arthritis ? | 57.8 | 56.8 | 1.0 | 70 |
| N 16 | F | 45 | Disloc. ankle | 57.0 | 57.0 | 0.0 | 0 |
| N 17 | M | 35 | ... | 58.0 | 58.0 | 0.0 | 0 |
| N 18 | M | 27 | Acute lumbago | 56.8 | 58.2 | -1.4 | - |
| N 19 | F | 61 | ... | 56.0 | 57.5 | -1.5 | - |
| D 20 | F | 69 | 10 years | 57.3 | 57.4 | -0.1 | 0 |
| D 21 | M | 70 | ... | 57.0 | 55.4 | 1.6 | 150 |
| D 22 | F | 76 | Cardiac failure | 58.4 | 58.2 | 0.2 | 20 |
| D 23L | F | 66 | 3 months | 60.2 | 61.4 | -1.2 | - |
| D 24L | M | 67 | 3 years | 59.0 | 60.4 | -1.4 | - |
| D 25L | F | 56 | 2 years | 56.8 | 60.1 | -3.3 | - |
| D 26L | F | 68 | 11 years | 56.4 | 59.4 | -3.0 | - |
| D 27L | F | 47 | 14 years | 54.0 | ? | ? | - |
| P 28 | F | 37 | 37 weeks | 56.3 | 54.1 | 2.2 | 380 |
| P 29 | F | 21 | 38 weeks | 57.0 | 54.4 | 2.6 | 350 |
| P 30 | F | 22 | 35 weeks | 56.8 | 54.6 | 2.2 | 190 |
| P 31 | F | 27 | 24 weeks, twins | 57.8 | 55.0 | 2.8 | 500 |

N: normal. D: diabetes. P: pregnancy. L: samples from Launceston.
 unmarked samples from Hobart. Treatment:- 1L X-Ray. 20, 22 insulin.
 13 pentothal. 18 sodium salicylate. 20 phenobarbitone.

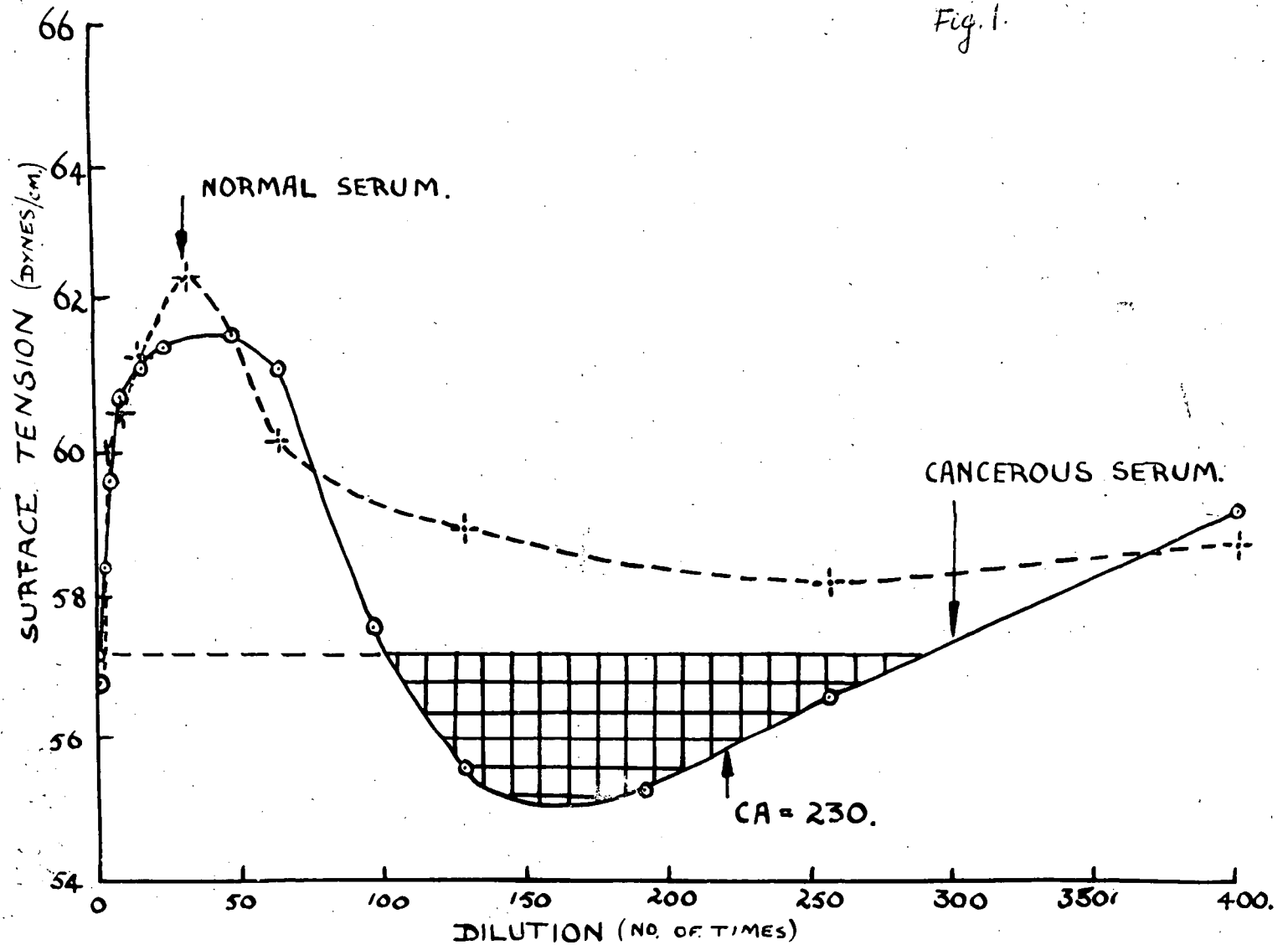
TABLE 3.

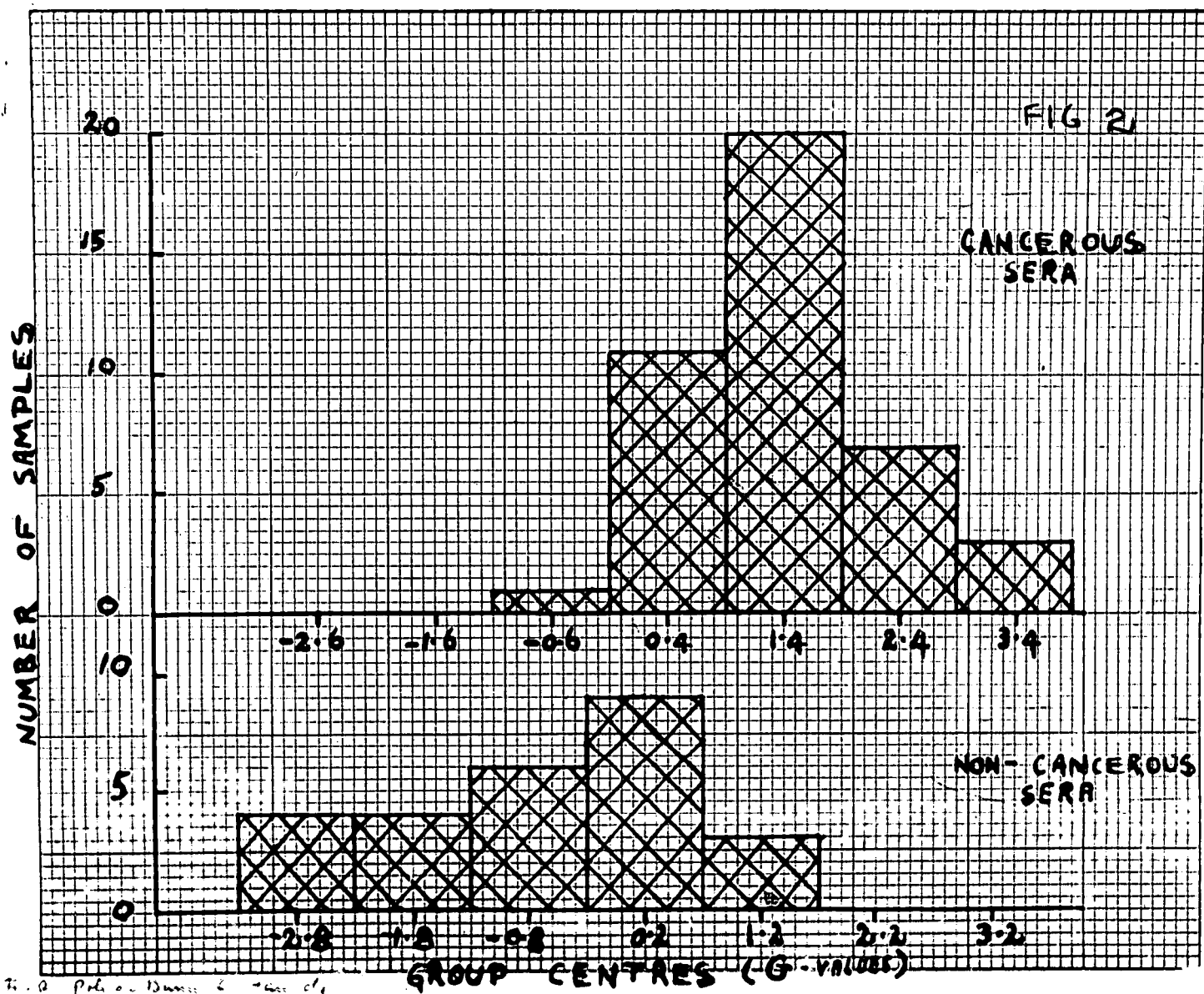
| <u>Origin</u> | <u>Delay</u> | <u>Average Ca</u> | <u>Average G.</u> |
|---------------|--------------|-------------------|-------------------|
| Hobart | - | 320 | 2.0 |
| Launceston | 24 hours | 210 | 1.1 |
| Sydney | 48 hours | 130 | 0.9 |

TABLE 4.

| | <u>Average CA</u> | <u>Average G.</u> | <u>Cases</u> |
|---------------------|-------------------|-------------------|--------------|
| Stomach, oesophagus | 330 | 1.5 | 4 |
| Uterus, ovaries | 250 | 1.7 | 11 |
| Breast | 220 | 1.3 | 7 |
| Neck, throat, mouth | 220 | 1.0 | 9 |
| Head | 180 | 1.3 | 4 |
| Lower Intestines | 180 | 0.9 | 3 |

Fig. 1.





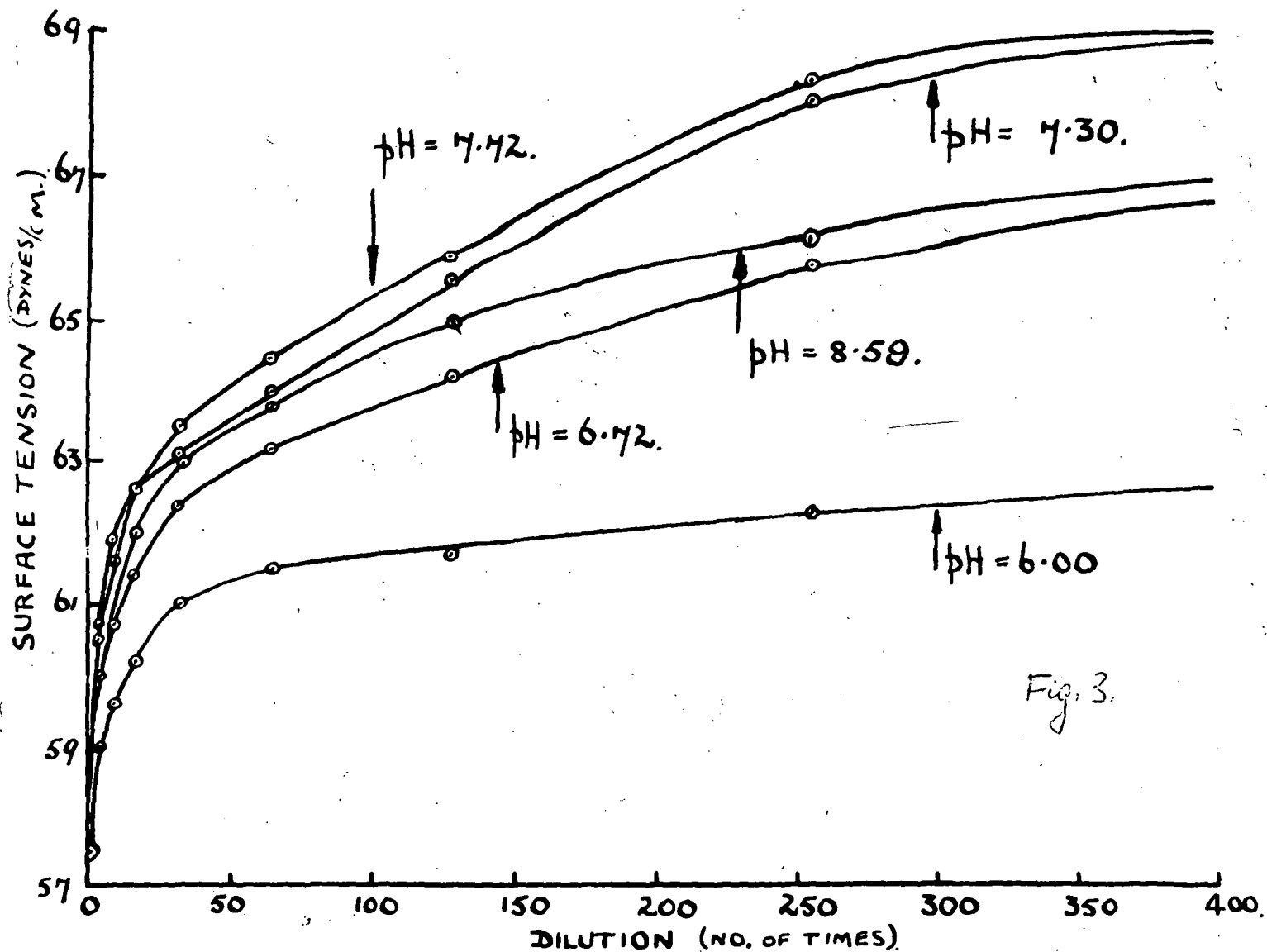
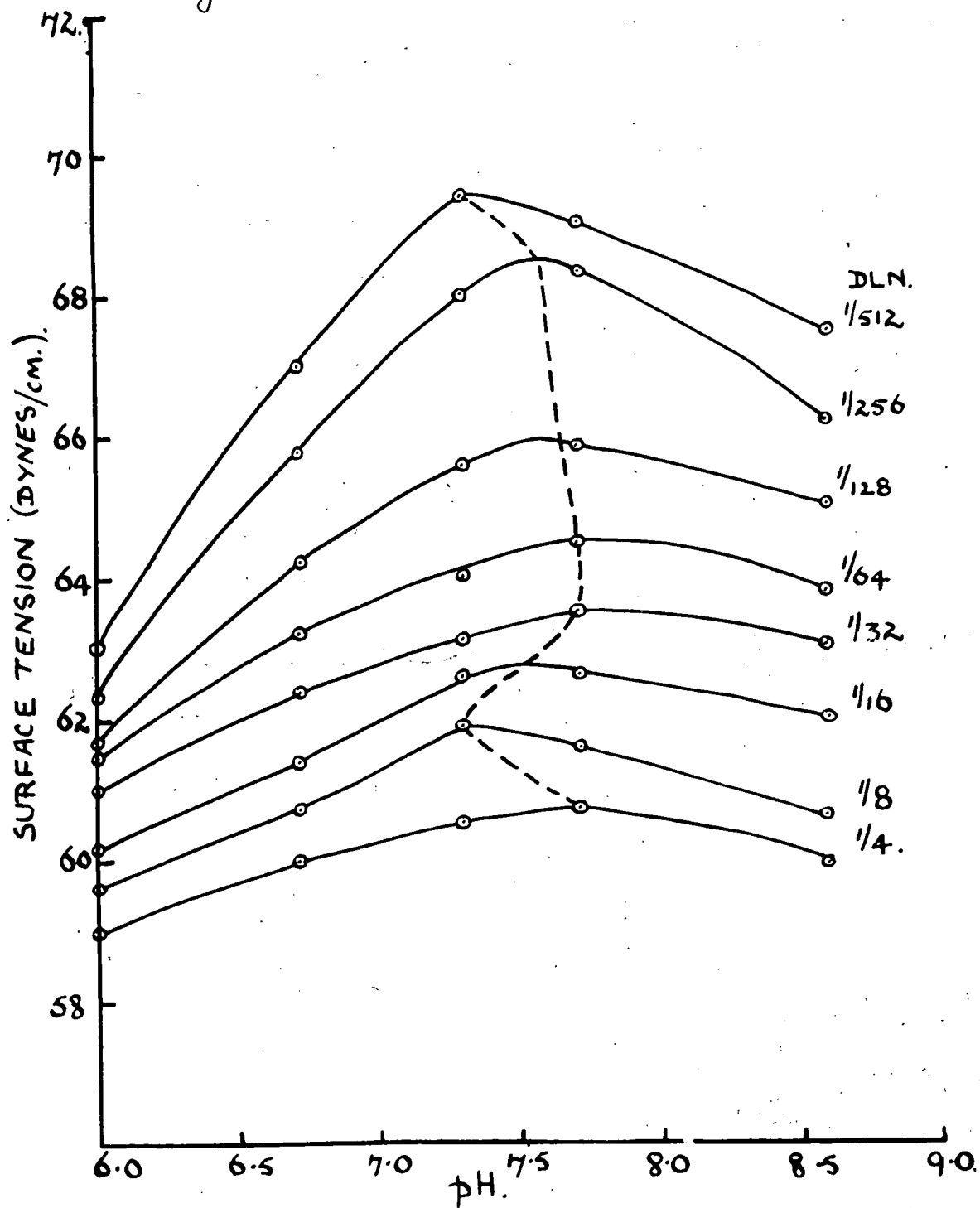


Fig. 3.

Fig. 4



Cytological Effects of 1,2,4-Triazoles I. The Action of 1-Phenyl-3,5-dimethyl-1,2,4-triazole on Roots of Allium cepa.

by W.D. Jackson (Botany Dept., University of Tasmania)

and

J.B. Polya (Chemistry Department, University of Tasmania)

(1) Introduction

The most important application of studies on the borderline of biology and chemistry is the chemical control of growth, in particular the chemical suppression of weeds, pathogenic micro-organisms and malignant cells. Studies in these fields are interrelated but the present work was stimulated by our particular interest in the latter two fields. Some compounds and classes of compounds have been found to be effective in the control of particular kinds of growth. Although such effects are denied or unknown for the great majority of chemical substances, the distinction between activity and inactivity in respect of growth inhibition appears to be mainly quantitative if results alone are taken in consideration. If the mode of action is chosen as the basis of classifying growth controlling substances, the differences between various agents are more readily defined, although even in this case the definition of growth controlling substances must remain substantially a pragmatic one. On surveying the available evidence, which is accumulating at a fast rate, one finds that the growth controlling substances act through one or more of the following mechanisms:

- (i) Competition with enzymes, coenzymes and essential structural units which may reduce synthetic activities in cells, lead to the formation of unsuitable structural units or an accumulation of toxic products.
- (ii) Destruction of enzymes, coenzymes and structural units required for growth.
- (iii) Alteration of physical conditions (permeability, pH, surface tension, viscosity, electro-magnetic properties, state of aggregation etc.) followed by the dislocation of biochemical balances either through an undesirable shift in reaction rates and energy relations or by permitting anabolic and katabolic processes to occur at undesirable sites.

These three mechanisms are not independent. Furthermore purely physical effects (heat, irradiation with ultra-violet light or X-rays, ultrasonic waves, cold etc.) may introduce their action through the third mechanism followed eventually by chemical effects of the first two kinds.

The exact mechanism of the action of mitotic poisons is unknown but the following general principles are probably valid.

- (1) The key compounds in mitosis are nucleic acids.

Their reactions during mitosis include (14, 15, 25, 42, 43).

- a) Attachment to and detachment from proteins;
- b) polymerization and depolymerization;
- c) equilibrium between deoxyribonucleic acid and ribonucleic acid.

The rebuilding of normal nucleic acids to ones with

reduced purine and increased phosphorus content is a feature of malignant cells. This may occur also in normal cells to limited extent but no detailed information is available on this subject. On the other hand it is unknown whether the rebuilding of nucleic acids in malignant cells is connected with mitosis or whether it is a secondary process occurring in the cytoplasm. In any case, substances capable of interfering with reactions a) -c) are expected to exhibit antimitotic properties.

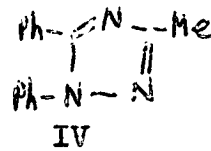
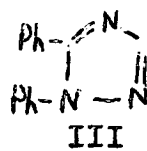
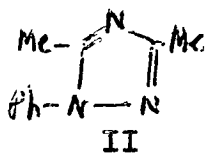
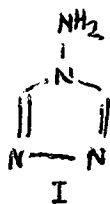
- (ii) In normal mitosis the proteins carrying the nucleic acids must conform to exact specifications concerning the structural and stereochemical identity of their component amino-acids, the linear and steric arrangement of these amino-acids, the grade of polymerization and the nature, number and site of prosthetic groups including the nucleic acids (12). Interference with any of these requisites is expected to disturb the normal mitotic process.
- (iii) The storage and transfer of energy liberated or drawn upon in the mitotic process depends on the formation and cleavage of high-energy phosphate bond (40). It should be possible to influence the course of mitosis by agents capable of stimulating, inhibiting or reshunting the phosphate metabolism within the nucleus but the general significance of energy transfers through phosphate bonds would render such experiments unspecific and would create serious systemic effects.

The introduction of 1,2,4-triazoles as cytological agents.

The problem of growth control was considered at first from the aspect of small chemical and/or physical alterations of nuclear proteins by simple organic substances. Simple amides like acetamide and urea are mild denaturing agents (44). They can penetrate cells without difficulty and have low toxicities. They are not useful antimitotics, but the fact that acetamide (but not urea) shows greater reactivity with cancer serum proteins than with normal serum proteins (38) suggested that derivatives of acetamide might be found with a greater differential effect. Diacetimide has approximately the same denaturing power as acetamide. Its effect on normal and cancer serum proteins is greater than that of acetamide but the experiments are difficult to duplicate owing to the instability of diacetimide in aqueous solution. Diacetimide has a slight narcotic effect on rats (Prof. F.M. Shaw, Department of Physiology, University of Melbourne). In the experiments of one of us (J.B.P.) it inhibited alcoholic fermentation temporarily only. Diacetimide, acetyl propionamide, dipropionimide, monochloro-diacetimide, symm. dichlorodiacetimide, chloroacetyl propionamide, acetyl benzamide, propionyl benzamide and chloroacetyl benzamide (39) do not inhibit Cl. welchii and Bact. coli in concentrations below 11/5000 (Prof. S.S. Kubbo, Department of Bacteriology, University of Melbourne). The formic hydrogenylase of Aerobacillus polymyxa is inhibited by chloroacetyl propionamide (6%), symm. dichlorodiacetimide (68%) and chloroacetyl benzamide (75%) (Biochemistry Section, Division of Industrial Chemistry, C.S.I.R.O.). The cytological effects of the fluorescent

halogenated diacylimines are not characteristic (Prof. H.M. Barber, Botany Department University of Tasmania). The observed toxic effects, however slight, could be due to chloroacetic acid since these compounds are not very stable to hydrolysis even in the cold. However the low solubilities in water make this explanation rather improbable. It is more likely that the halogenated compounds react directly with proteins in view of their similarity with nitrogen mustards. Again it was considered that acetamido and diacetimido react with proteins in the absence of halo_gen functions which suggested experiments on further derivatives of amides and diacylimines.

As a first step in this programme a number of 1,2,4-triazoles have been prepared partly because some of them are synthetically derived from diacylimines, partly because all 1,2,4-triazoles may be considered to consist of two amidine structures fused within a system of appreciable resonance energy and some high resonating amidines (e.g. paludrin) have aroused much therapeutical interest in recent years. 4-Amino-1,2,4-triazole (I) has been prepared by a published method (2). The 1-phenyl-3,5-dimethyl (II), 1,5-diphenyl (III) and 1,5-diphenyl-3-methyl (IV) derivatives of 1,2,4-triazole have been prepared in good yields by a convenient modification of the Brunner reaction (11) details of which will be published elsewhere.



Various acylated hydrazines have been obtained as byproducts. None of these showed significant cytological effects. This suggests that the effects observed with 1,2,4-triazoles are not primarily due to their derivation from hydrazines. I and II are readily soluble in water while III or IV are little soluble at pH values higher than 6. For this reason and others to be explained later the effect of II only was investigated in detail. The preparation of a series of triazoles is in progress and we propose to report on their cytological and other biological effects from time to time.

Properties of II in relation to biological effects.

a) II is soluble in 7 parts of water at room temperature while III and IV require more than 1000 parts of water for solution at pH values above 6. On Ostergren's theory (32, 36) one would expect stronger c-mitotic effects with III and IV than with II. Although the observed cytological effects may not be regarded as pure c-mitotic effects and the quantitative share of the c-mitotic effect can not be estimated at present, it appears that the differences between the c-mitotic activities of the triazoles are not as great as those between their solubilities in water.

b) Molten II at 46-50° dissolves desoxyribonucleic acid from thymus and crude nucleic acids prepared from yeast. Contrary to expectation, the presence of nucleic acids in molten II enhances spontaneous crystallization on slow cooling to room temperature. In the absence of nucleic acids even the purest preparation of II require chilling or seeding to promote crystallization at a comparable rate. Slightly

discoloured solutions of desoxyribonucleic acid in cold buffer solutions of pH 7 to 8 become lighter on the addition of II. This effect is less pronounced, and possibly not significant, in the case of crude yeast nucleic acids.

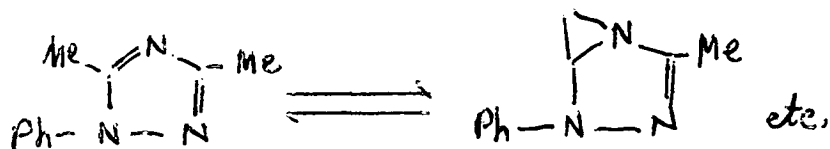
Nucleic acids dissolve in aqueous 5% solutions of II. The Feulgen reaction of such solutions is weaker than that of solutions of identical concentrations of nucleic acid in sodium acetate. The cytological observation of "nucleic acid starvations" in cells treated with II is in agreement with this finding. Work is in progress (with A.E. Parkes) on the quantitative chemical and physical details of the interaction of II with nucleic acids.

c) Work in progress (with A.E. Parkes) indicates that II interacts with serum proteins. With II : protein ratios of the order of about 100, the interaction (followed by surface tension measurements) is the same with both normal and cancer serum proteins, but significant qualitative and quantitative differences occur at higher ratios. Treatment with II hardens the cells in a similar manner as treatments with formaldehyde or heavy metal salts, although the degree of hardening is less in the case of II. Other cytological changes, collectively termed as "stickiness" are similar in the cases of treatment with II and heavy metal salts (17). Spindle suppression makes it plausible to assume that the denaturing action of II is particularly strong on the centromere.

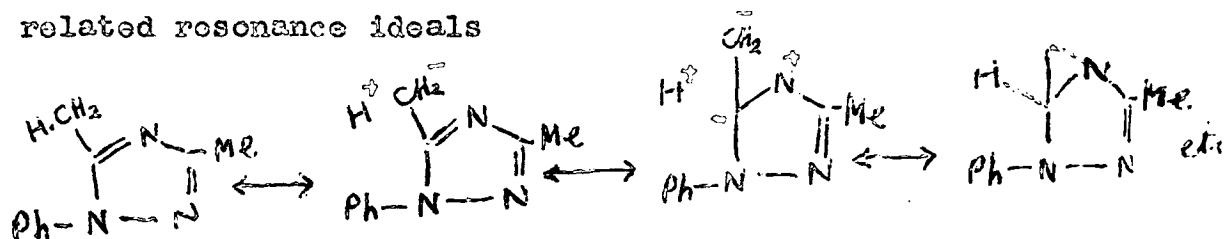
d) Even the purest preparations of II undergo some auto-polymerization with discoloration on standing. Fresh preparations are brown when fused. On cooling the colour becomes

lighter and the liquid solidifies to white crystals. Finally the catalytic effect of light on the polymerization of II point to the ready formation of free radicals. These effects are less marked in the case of diphenyltriazoles and thus it is possible that there is some connection between polymerization and antimitotic action. The anomalies of the physical properties of some simple heterocyclic compounds, including 1,2,4-triazoles (27), are similar to those of the amides which suggests that these compounds exist in molecular organizations which are inferior yet akin to those of proteins. While the interactions of simple molecules are well understood, interaction between "super-molecules" (i.e. molecular systems) has not been studied extensively apart from ^{immune} logical investigations. It is felt that research into the interaction between highly organized super-molecules, like proteins, and super-molecules of a lower order of organization, like amides, 1,2,4-triazoles, imidazoles etc., would considerably simplify the difficulty in establishing a general theory of super-molecular reactions. The fact that comparatively brief investigations into the effects of simple amides and triazoles, have yielded interesting results may be of heuristic significance.

e) II may be compared briefly with some recently described antimitotic poisons like polyepoxy compounds and ethylenimine derivatives the effects of which may be due to free radical mechanisms (41). In the case of II one could consider tautomeric forms



and related resonance ideals



which suggest some similarity with ethyleneimine derivatives and, possibly, nitrogen mustards (13). The superiority of II over other 1,2,4-triazoles in which the 3- and 5-methyl groups are partly or wholly replaced by hydrogen or phenyl groups lends some support to the view that at least part of the cytological action of II could be an "ethyleneimine effect". The lack or scarcity of chromosome breakages on treatment with II, which is still in doubt, would suggest the opposite unless one considers that while free radicals are always likely to interfere with the normal mitosis through the modification of proteins and nucleic acids involved in the mitotic process, there is no proof at present for the axiom that all free radicals must cause chromosome breakage. Furthermore the nature of the H- substituents of ethyleneimine derivatives must modify the cytological effects to a considerable extent leading to chromosome breakage in some cases and to less drastic effects interfering with normal mitosis in others.

Triethylene melamine polymerizes at 70°C at a pH of 7.0. It reduces the swelling and water adsorption of regenerated cellulose (1), and has promising anti-mitotic properties (43). If it is correct to regard the antimitotic effects of II as related to those of triethylene melamine, the chromosome

contraction caused by the latter could be explained by a similar dehydration of colloid elements.

Experimental procedure.

Bulbs of Allium cepa with young roots were immersed in double serial concentrations of the triazoles in water at 14 - 18°C. At intervals (4 hour multiples) roots were removed and fixed in acetic-alcohol 1:3, or in Navashin fluid for 24 hours at 14-18°C. (28-30, cf. 22). Where toxic effects were indicated by transparency or lack of turgor, bulbs were transferred, with thorough washing, to tap water for recovery. During recovery root tips were removed and fixed as above.

Root tip material fixed in Navashin fluid was embedded in paraffin, section at 16 μ and stained in crystal violet or in haematoxylin. Material fixed in acetic-alcohol was treated by squash methods with aceto-orcein, or the Feulgen differential stain (28-30, cf. 5, 6, 15, 24).

Control bulbs were grown in tap water and in aqueous 0.05% colchicine solution (31, 32). Root tips used for controls were removed at the same time as treated roots in order to separate cyclic fluctuations in the division rate from changes induced by treatment with triazoles. Control root tips were placed in muslin bags and processed together with treated root tips in order to exclude variations in depth of staining, and degree of spiralization, inherent in fixation and staining.

Slides were photographed using contrast filters on 9 cm x 6 cm Kodak ortho plates at magnifications of 500 x and 1000x. Squash preparations were edge sealed and

1.
and photographed before being made permanent in euparal.
Camera lucida drawings were made using a 1/12" 95x oil
immersion objective and a 20x eyepiece.

Observations

I in 1% aqueous solution for 16 hours produces severe toxic effects from which recovery is not possible. Recovery was possible after 4-8 hours' treatment without significant physical or structural alterations with the exception of ragged nuclei and understaining in Foulgen stain. Weaker concentrations first stop division, then cause death. II in a concentration of 1% immediately suppresses division and recovery becomes impossible after 16 hours' treatment. Concentrations of 0.5% cause toxic effects from which recovery is possible after 24 hours but not after 28 hours. After 16 hours' treatment the rate of division approximates zero and toxic effects are evident. After 8 hours' treatment the rate of division is low but toxic effects are not apparent. The low rate of division in the phase of increasing toxicity makes it difficult to find the number of divisions necessary to establish the effect of the treatment. Treatment with a concentration of 0.1% produces slight spindle disorganization and the rate of division approximates zero after 48 hours of treatment. Bulbs exposed to this concentration for 2 months are not killed but growth in length of the roots is suppressed and slight tumours in the elongation regions are formed. Concentrations lower than 0.1% show little effect during the first 3-5 days of treatment but the rate of growth is considerably reduced on longer treatment.

Roots recovering from 4-24 hours' treatment with 0.5%
II show the following effects (Plates 1 to 8):

a) Resting stage.

4-8 hours: Numerous bridge binucleate and other multinucleate cells containing micro-nuclei. Extended periods of recovery show that bridge binucleates tend to be formed even after 24 hours of recovery.

8-12 hours: The numbers of bridge binucleates and multinucleate cells do not increase but single nuclei of ragged appearance, more weakly and unevenly stained, become common. Extended periods of recovery produce a sudden increase of the incidence of bridge binucleates which continue to be formed for up to 4-5 days.

12-24 hours: Misshapen nuclei with increasing tendency of weaker Feulgen staining; increase in the size of nucleoli. In longer treatments a granulation effect in the cytoplasm is noted.

b) Prometaphase

The chromosomes are unevenly stained and less spiralized. A precocious separation of the chromatids is especially marked in recoveries from treatments of over 8 hours.

c) Metaphase

4-8 hours: The chromosomes are markedly contracted (down to below 50% of the control lengths in the case of 8 hours' treatment). There is little or no organization to a metaphase plate with the consequent dispersed effect of treatment with colchicine. There is a transient tendency to form ball and exploded metaphases.

8-12 hours: The contracted chromosomes are dispersed in the cell. The entire blocking of the metaphase decreases

with increasing recovery. The chromosomes are weakly and unevenly stained with a vesicular appearance in the chromatids. Recovery shows some cells with possible true diplochromosomes of 4-partite structure.

12-14 hours: All divisions are entirely blocked at metaphase. Ragged reversion states are common.

d) Anaphase.

4-8 hours: There are scattered chromatids and lagging with marked failure of some chromosomes to divide at the centromere. This gives rise to some X structures typical of colchicine treatment but most chromatid arms remain parallel as in the case of treatment with sulphanilamide (39).

8-24 hours: Where the blocking of the metaphase is not complete the chromatids are dispersed at random and there is no orientation to the poles. Recovery restores normal spindle action rapidly but bridges at anaphase persist for several days. These bridges appear to be confined to sister chromatids although several doubtful cases of bridges between other chromatids have been observed. Owing to the similarity between the chromosomes of Allium cepa, detection of such cases is difficult. Bridges seem to be confined to terminal and near-terminal regions and give rise to peculiar "pseudo-chiasmata" (Diagrams and Plate 9). The contracted chromatids are weakly and unevenly stained and have a regular vesicular structure which appears to be loose spiralization. (Plate 10).

e) Telophase.

Lagging results in ragged shapes. Varying numbers of bridge connections are observed. The cell plate initiation

fails or is delayed.

III in a saturated solution slows down the lengthwise growth of roots but shows no toxic effects after 1 month of treatment. The rate of division is not appreciably altered over a period of several days but a tumour of the root appears after 4 days' treatment. During further growth this portion of the root has a diameter about 25% larger than the original diameter. Cells in this region show a lack of elongation. Divisions appear to be quite normal. Tetraploid cells appear in each root but these do not appear to be more common than what is normally expected (8).

IV in a saturated solution has the same effects as III although the inhibition of lengthwise growth is somewhat stronger.

Discussion

The weaker effects of III and IV in comparison with I or II may be due to the low solubility of the former in water. It is proposed therefore to extend these investigations to tests in acidic buffer solutions. We also propose to report on the cytological effects of a larger series of triazoles in due time. For the time being the discussion is restricted to II although this will cover to some extent I, III and IV the cytological effects of which differ from those of II quantitatively rather than qualitatively. It may be considered that II exhibits the following four effects:

a) At low concentrations mitosis is inhibited without toxic effects apart from a slight contraction of the chromosomes. The spindle action is strong and the rate of division increases to normal during recovery.

b) Treatment with concentrations of 0.5% for short periods produces a powerful c-mitotic effect with a rapid and complete breakdown of the spindle action. The threshold range of this effect lies close to that of severe toxicity and can be demonstrated during recovery only. The most readily noted phenomenon under this heading is the contraction of chromosomes to less than 50% of the control lengths. Otherwise the c-mitotic effect in its early stages resembles that produced by benzene vapour (9) and in later stages that produced by sulphanilamide (37). As in sulphanilamide treatment, the chromosome arms do not exhibit the repulsion between chromatids which leads to the typical X structures on treatment with colchicine. The chromatid arms lie parallel until division of the centromere or reversion occur. Multipolar effects are not found and the chromosomes are lightly and unevenly stained.

The physical condition of the chromosomes at blocked metaphases indicates that reversion to the resting stage occurs without division of the centromere. Treatment with colchicine (26) or sulphanilamide (27) gives a similar effect which, however, is quantitatively less significant than in the case of treatment with II where this kind of reversion is more common than reversion after complete division. Owing to the much depressed rate of division detection of diplochromosomes or of polyploid cells in the following recovery period is difficult. Hence it is not quite certain whether division occurs during reversion stages.

During early stages of treatment and late stages of recovery division of the centromere occurs. Where the spindle action

is weak or absent either tetraploid nuclei result or the scattered chromatids undergo reversion to form multinucleate cells. Where the spindle action is stronger there is evidence of ball and exploded metaphases. Reversion from this stage is again direct.

c) A feature of the recovery is the common occurrence of chromatid bridges and "pseudo-chiasmata" (Diagrams and Plate 9) at anaphase. These continue to be formed for several days after treatment. Telophases with several bridges are not uncommon even though the spindle action has returned to normal and the division rate is increasing. Bridges could be the result of reunion with or without breakage. However only a few doubtful cases of fragmentation have been observed and these are likely to have been caused by the mechanical rupture of stretched bridges. Hence fragmentation in the normal sense may be excluded. Since chromatid fusion and sister reunion, if possible, without breakage, have only been demonstrated between chromatid ends, it seems unlikely that a mechanism is applicable in this case since many of the bridges are formed in a near but non-terminal position. It is suggested therefore that bridges result from either the failure of certain portions of the chromosomes to reproduce or to intercalary stickiness resulting from some surface phenomena. Reunion of sister chromatids has been shown to occur in aged seeds (34) and pollen grains (3). Structural changes due to ageing have recently been brought to notice in Allium cepa seeds used in experiments by Barnard (7). A closer study of this phenomenon may reveal similarities between the effects produced by enzy-

natic changes during ageing and those evoked by antimitotic agents like the one under consideration.

The stickiness produced by II results in features (cf. Plates 9 and 10) which are practically identical with those produced in Allium cepa by colchicine and cold (D'Amato (17, 18)). Low temperature effects have been studied by Darlington and La Cour (21) and Barber and Callan (4).

It is also of interest that stickiness is known to exist as a recessive in Zea mays (10). Darlington has advanced the theory that stickiness induced by cold is the result of nucleic acid starvation, which results in misdivision of terminal or near-terminal chromosome material (19, 20, 21, 23).

In the case of II some evidence has been advanced that it reacts with nucleic acid and that there may be a gradual loss of of desoxyribonucleic acid on prolonged treatment as shown by the gradual weakening of the Feulgen staining. The interaction of II with proteins introduces another possibility to explain stickiness but it is probable that the effects of any physical or chemical antimitotic agent on nucleic acids and proteins of the cell are not independent phenomena. Ostergren (35) has shown that ethylene glycol produces stickiness in 0.25-41 concentrations but in the absence of data on recovery from such treatment one may not regard as proved that stickiness is due to factors other than slow death in that case which is quite different from that of treatment with triazoles recovery from which still permits the observation of stickiness. A number of other compounds causing breakage and bridge formation have been reviewed by Loveloss and Revell (33) who ascribe these effects to intercalary stickiness resulting from cross-linkages.

Allium cepa is not the best material to observe breakages on treatment with triazoles. *Tradescantia* sp. ($2n = 12$) was used in some experiments with II which could be introduced into the inflorescence by standing the end of the cut stem in a 0.5% solution. Various blocked meioses and first pollen grain mitosis were observed. There is also interference with the differentiation pattern within pollen grain, an effect which can be produced by heat treatment. The generative and tube nucleus are randomly oriented and differentiation is not marked. On preliminary evidence the appearance of fragments may be more common than in Allium cepa.

d) At greater concentrations than 0.5% a general toxic effect appears which becomes very powerful at 1.0%. The increase of toxicity is very steep at higher concentrations which may indicate severe denaturation of cell proteins.

Applications of II.

The rapid and total suppression of spindle formation and the contraction of chromosomes by II suggest that it could be used in fast squash methods to facilitate the counting of chromosome numbers of species with long chromosomes. Thus the chromosome numbers of the Eulbine and Arthrotaxis species have been determined in a few hours after treatment with a 0.5% solution of II whereas the normal paraffin method requires considerably more time and labour and is unreliable.

The toxicological effects of II and III were tested by Prof. F.H. Shaw (Department of Physiology, University of Melbourne) with whose permission the following preliminary findings are communicated. Intravenous injection of II in rabbit slows the heart rate and increases the respiratory rate;

300 mg/kg is an almost fatal dose. About 50 mg/kg of III causes muscular spasms and is an almost fatal dose. A wild rabbit tolerated daily intraperitoneal injections of 0.5 g. II for two months without ill-effects, then suddenly its temperature began to drop, breathing and pulse became slow and death occurred a day after developing these symptoms. The post-mortem showed normal liver and lungs. The heart was considerably distended. The abdomen contained much fluid. On the internal surface of the abdominal wall and some parts of the gut there were numerous small haemorrhages which extended up to the level of the diaphragm. At four places there was marked adhesion of the bowel to the abdominal wall. The adhesions were tough and covered large areas. The kidneys were almost globular and were very tough to cut they had normal colour and showed no gross internal changes. The left kidney adhered strongly to the rear abdominal wall.

The action of II on a pure culture of brewer's yeast was studied with Miss E. Ashbolt. Details of this work will be reported separately but the following observations may be of interest in connection with the present work. On treatment with II young cultures of yeast acquire the morphological characteristics of old cultures. Fermentation is inhibited at II concentrations of 0.25% and more.

Summary

- 1) 1-Phenyl-3,5-dimethyl-1,2,4-triazole has four well defined but overlapping effects on the root of Allium cepa:

- a) While recovery is still possible after 2 months treatment at 0.1% concentration, treatment for 24 hours is sufficient to reduce the division rate

to nearly zero and to hold it at this level in recoveries for several days.

- b) c-Mitotic effects induced at low concentrations but better studied at intermediate concentrations, ~~these~~ include tumor formation, rapid and extensive suppression of spindle action, and a high degree of chromosome contraction. Polyploidy in cells is difficult to detect owing to the low division rate. This effect resembles that of benzene vapour or sulphanilamide.
 - c) Intercalary sticking between chromosomes at anaphase is observed during recovery and is not due to slow death. The understaining in Feulgen stain, the probable decrease in spiralization and larger nucleoli suggest interference with the nucleic acid cycle.
 - d) Prolonged treatment or high concentrations lead to granulation in the cytoplasm and other general toxic effects.
- 2) Under the investigated conditions 4-amino-1,2,4-triazole is more toxic and 1,5-diphenyl-1,2,4-triazole and 1,5-diphenyl-3-methyl-1,2,4-triazole less effective than 1-phenyl-3,5-dimethyl-1,2,4-triazole.
 - 3) In animal experiments 1,5-diphenyl-1,2,4-triazole is considerably more toxic than 1-phenyl-3,5-dimethyl-1,2,4-triazole.
 - 4) 1-Phenyl-3,5-dimethyl-1,2,4-triazole is superior to colchicine as an auxiliary reagent in squash method for the counting of chromosome numbers.

5) Attention is drawn to possible correlations between the chemical and physical properties and the cytological effects of 1-phenyl-3,5-dimethyl-1,2,4-triazole. The denaturing proteins and the deterioration of desoxyribonucleic acid in aqueous solutions of 1-phenyl-3,5-dimethyl-1,2,4-triazole are noteworthy in this connection.

Acknowledgments.

The authors wish to acknowledge a generous grant from Mr. E.J. Hallstrom and are indebted to Professor H.N. Barber for his interest and advice and to Professor F.H. Shaw for toxicological tests. Thanks are also due to Dr. A. Komzak and Mr. M.R. Atkinson for their assistance with triazoles and to Miss E. Ashbolt and Mr. A.E. Parkes some of whose findings are included in this paper.

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PLATES AND DIAGRAMS

Plates 1 - 4 : 0.5% 1-phenyl-3,5-dimethyl-1,2,4-triazole treatment;
the c-mitotic effects increase from 1 to 4 . (500x)

- Plate 1 : 4 hours' treatment ; recovery 12 hours ;
slight contraction , normal spindle formation .
- Plate 2 : 8 hours' treatment ; recovery 8 hours ;
marked contraction , weak spindle action .
- Plate 3 : 8 hours' treatment ; recovery 12 hours ;
marked contraction , very weak spindle action .
- Plate 4 : 8 hours' treatment ; recovery 16 hours ;
super-contraction , no spindle action .

Plates 5 - 8 : illustrations of intercalary sticking . (500x)

Plates 5 - 7 : 0.5% 1-phenyl-3,5-dimethyl-1,2,4-triazole ;
8 hours' treatment ; recovery 24 hours .

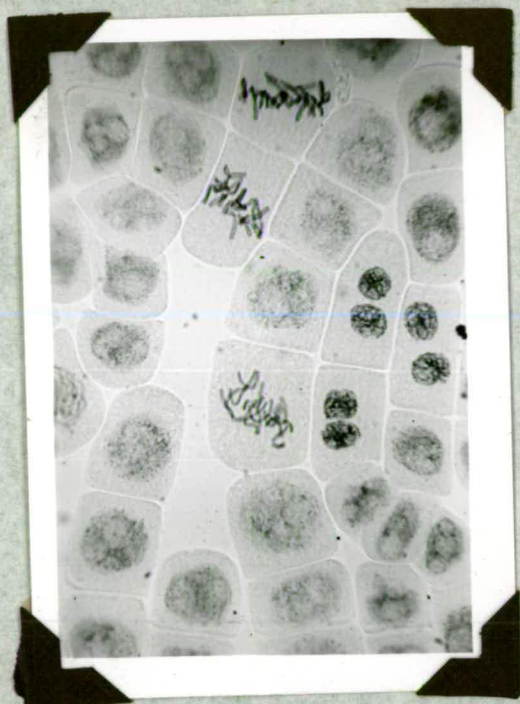
- Plate 5 : lagging and failure of division .
- Plate 6 : failure of division and intercalary sticking .
- Plate 7 : telephase bridges .

Plate 8 : 1% 1-phenyl-3,5-dimethyl-1,2,4-triazole ;
8 hours' treatment , recovery 12 hours . (500x)
reversion with and without division.

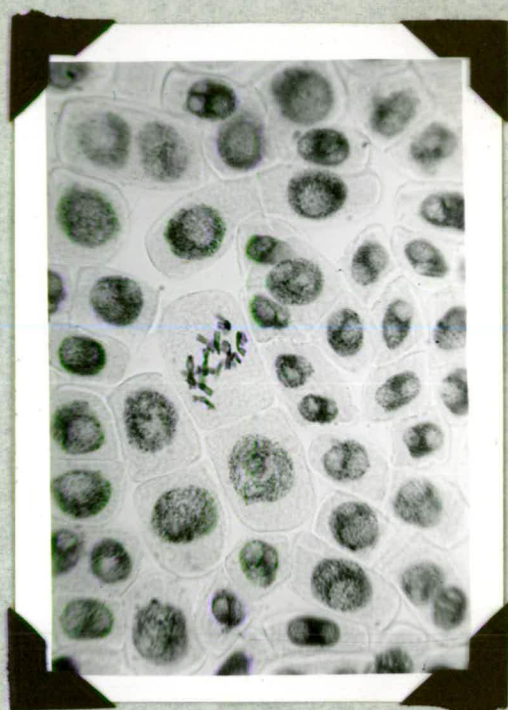
Plates 9 -10 : 0.5% 1-phenyl-3,5-dimethyl-1,2,4-triazole ;
8 hours' treatment , 48 hours recovery . (2000x)

Plate 9: intercalary sticking .

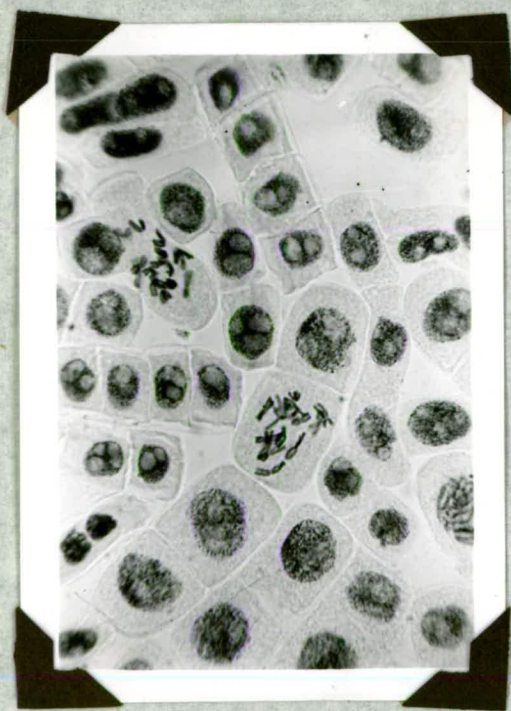
Plate 10: chromatid structure .



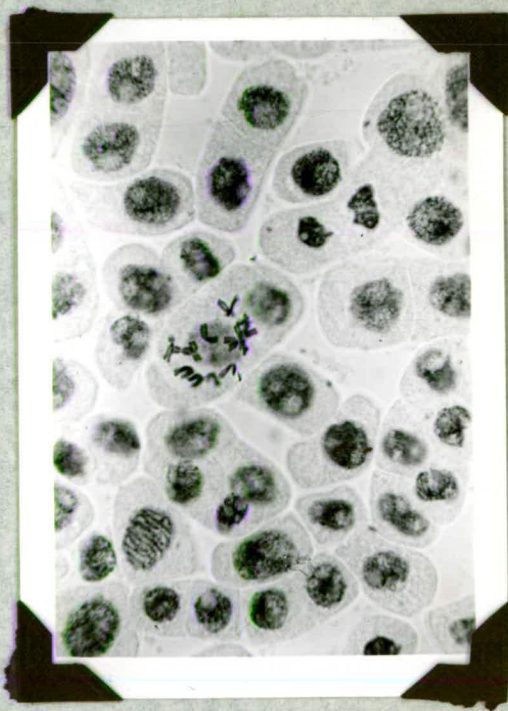
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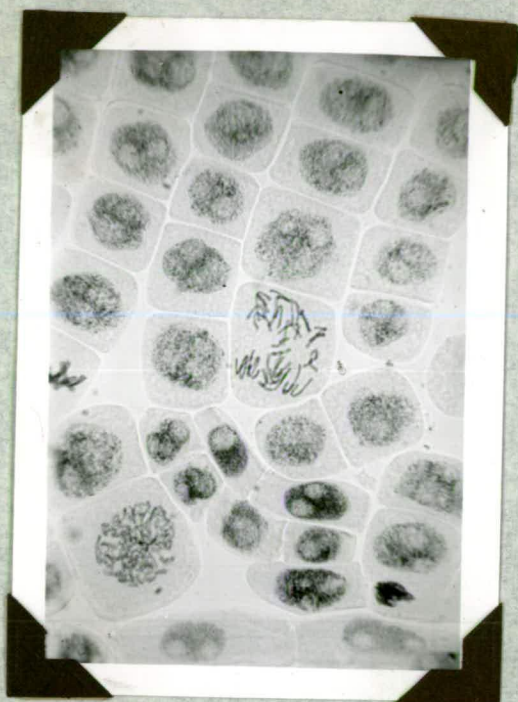
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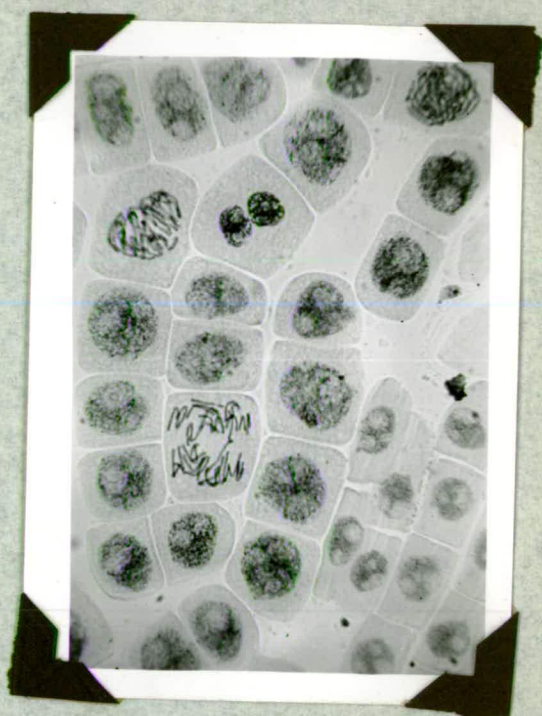
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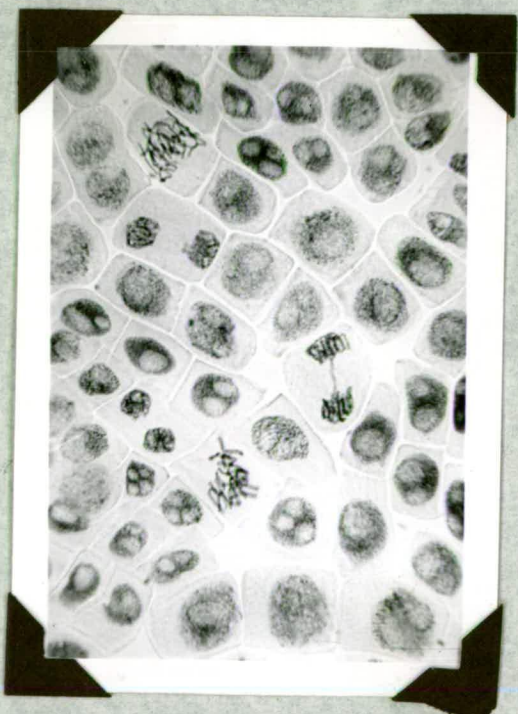
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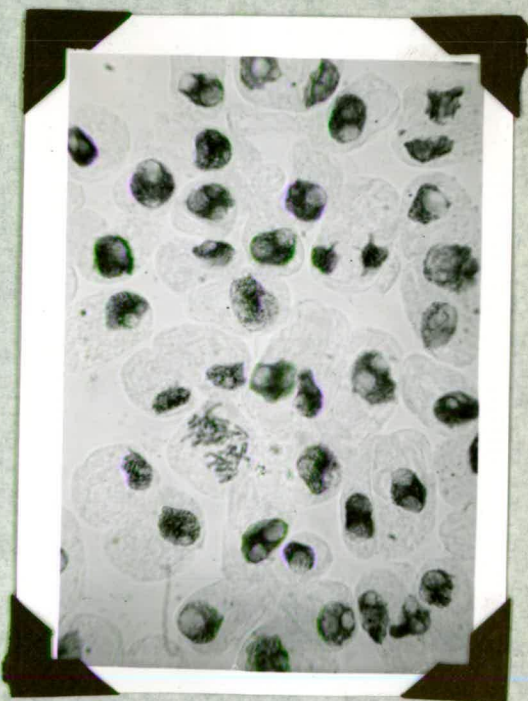
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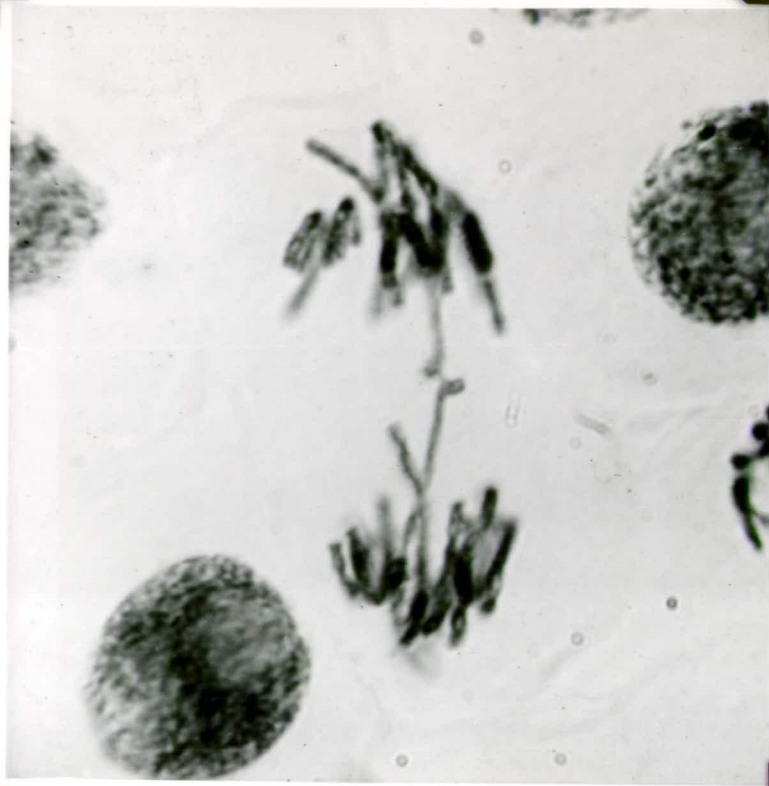


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8

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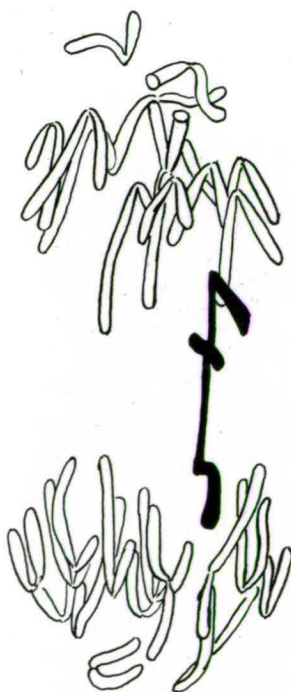
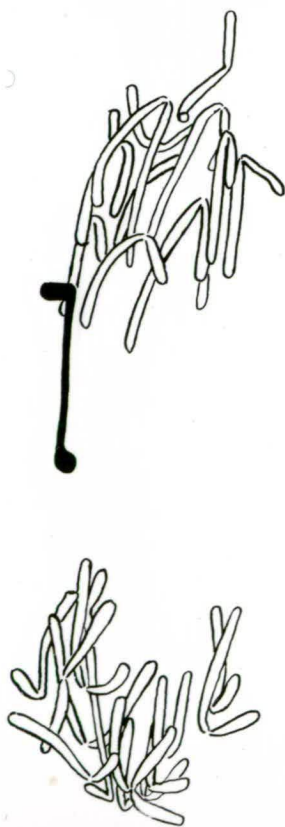
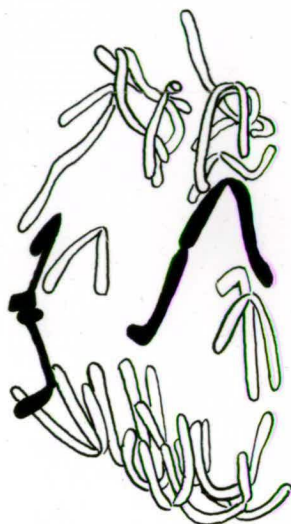
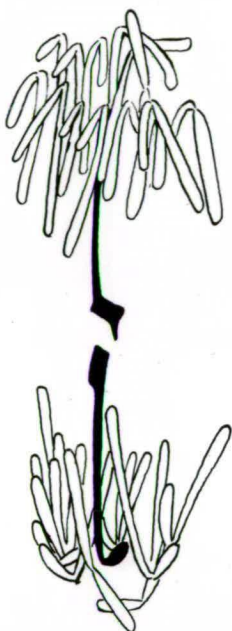


9



10

Diagrams : 6 anaphases showing intercalary sticking ;
0.5 % 1-phenyl-3,5-dimethyl-1,2,4-triazole ;
8 hours' treatment ; recovery 48 hours . (approx. 2000 x)



B. MISCELLANEOUS MINOR INVESTIGATIONS .

The following papers are submitted in this section :

- 1) J.B.POLYA, N.K.VALLANCE : Notes on the Chemistry of Alkali Lignin. - Roy.Austr.Chem.Inst.J. , 1950 , 17 , 353 . - Reprints are not yet available .
- 2) Notes on Organo-cobalt Compounds . - This material is being prepared for publication with D.A.E.Briggs . It is based mainly on the Honours work of D.A.E.Briggs who carried out his investigations under the direction of the author .
- 3) A.D.DARGAVILLE , D.L.INGLETON and J.B.POLYA : The Recovery of Organic Material from Cobalt 1-Nitroso-2-naphtholate . - This paper has been received by the Editor of the Journal of the Society of Chemical Industry .
- 4) J.B.POLYA , B.WILSON : Colorimetric Micro-determination of Cobalt . - This paper has been set up in print by the Australian Journal of Science and is expected to appear in December 1950 .
- 5) The Preparation of 2-Nitro-4-aminophenylarsonic Acid . - This paper has been received by the Editor of the Journal of the Society of Chemical Industry .

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By J.B. Polya, D.Sc., Techn., F.R.I.C., F.A.C.I. (x) and
N.K. Vallance, B.Sc. (Hons.) (xx)

The extraction of various kinds of lignin with acetonitrile (1) is expensive and the theoretical interpretation of the anomalous lignin values of the extracts is difficult. It has been decided therefore to concentrate future efforts on the investigation of one kind of lignin at a time and to investigate more convenient solvents only. Alkali lignin precipitated from black liquor, obtained through the courtesy of Associated Pulp and Paper Mills, Burnie, has been selected for the first series of experiments. Attempts have been made to purify and/or to fractionate lignin by extraction with single solvents and with a succession of solvents. In particular the possibility of effecting some measure of separation between lignin corresponding to different degrees of methylation was investigated. Metallic and acyl derivatives of lignin were prepared. The formation of plastic materials from lignin and phthalic anhydride was investigated.

The optimum conditions for the precipitation of lignin from black liquor were investigated in experiments based on earlier work by Plunguian (2) and by the West Virginia Pulp and Paper Co. (3). The following methods were found to be most satisfactory:

Method 1: Black liquor is diluted with water to density 1.2 and warmed to 40°C. Carbon dioxide is passed through the vigorously stirred liquid until precipitation is complete and then for a further 15 minutes. After heating ^{to} 90°C. and rapid cooling to 40°C. the precipitate is filtered on a coarse paper

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under suction. The precipitate is washed with cold water (4 times the volume of the black liquor), transferred to a beaker and stirred with cold water (6 times the volume of the black liquor) for 30 minutes. After allowing to settle for 16 hours the bulk of the liquid is decanted and the precipitate is filtered under suction. The beaker is rinsed with cold water (half the volume of the filtrate) and the washings are poured through the filter. The combined filtrates from the last operations amount to nine times the volume of the original black liquor and contain 2 per cent. lignin as sodium lignate. The filtered precipitates may be converted to a material with 0.34 per cent. ash, consisting almost entirely of sodium salts by treatment with dilute sulphuric acid. Sodium lignate obtained by this method was used for the preparation of metallic derivatives of lignin.

Method 2: Black liquor is diluted to density 1.2 with cold water then to ten times this volume with boiling water. Sulphuric acid (2N) is added until effervescence ceases and the solution is distinctly acid to litmus. The precipitate is allowed to settle for 2 hours, the supernatant liquid is decanted and the precipitate is washed twice with hot water (90°C., 5 times the volume of black liquor in each washing), the washings being removed by settling and decantation on each occasion. Finally the precipitate is washed in a similar manner with three portions of cold water (volumes as before). The precipitate is sucked as dry as possible and air-dried at 37°C. for 4 days. If the material is very wet at this stage

it may set to a cake. In this case prolonged air-drying at room temperature and the use of porous plates is desirable before proceeding to drying at 37°C. The oven-dry material is ground, screened (60-80 mesh) and air-dried for a further 4-5 days.

Acidification of the sodium lignate solution obtained by Method 1 or of the precipitates obtained by the same method gave lignin identical with that obtained by Method 2. The material is dark brown, dense, flows freely and decomposes over a wide temperature range above 180°C. It gives 88 per cent. Klason lignin, and contains 14.50 per cent. methoxyl and 7.0 per cent. moisture. The solids in the sodium lignate solutions give 80.5 per cent. Klason lignin and contain 8.9 per cent. sodium.

Extraction experiments were carried out in Soxhlet extractors with 8 g. lignin and 200 ml. solvent for 5 hours. These figures refer to experiments with a single solvent. In experiments involving a succession of solvents the quantity of each new solvent and the extraction times remained as stated but the amount of extractable material in the thimble diminished at each stage of such experiments. Extractions were carried out with water, alcohols (methanol, ethanol, n-propanol, isopropanol, n-butanol and isobutanol), ethers (diethyl ether and dioxane), acetone, a mixture of equal volumes of methanol and acetone, benzene, and pyridine. Extraction with diethyl ether, dioxane and benzene gives similar materials but the solubility in the latter two solvents is very slight and a full investigation of

dioxane or benzene extracts was omitted. Pyridine gives an apparent extract of 112 per cent, with a nitrogen content of 7 per cent. The extract has a distinct odour of pyridine but does not lose nitrogen on prolonged drying at 115°C; it is a tough, rubber-like resin. A full investigation of this extract has not been possible yet but the authors hope to report on this matter at some later time. Yields and analyses of extracts with water, methanol, ethanol, ether, acetone and acetone-methanol are shown in Table 1. Comparative yields of extracts for water and alcohols are shown in Table 2.

TABLE 1
Lignin Extracted by Various Organic Solvents

| Solvent | Extracted % | Yield of Klason lignin | | Methoxyl Content of Extract | |
|------------------|----------------|---------------------------|----------------------------------|--------------------------------|----------------------------------|
| | | based on extract % | based on orig. lignin % | based on extract % | based on orig. lignin % |
| Water | 16.3 | 82.1 | 13.4 | 13.99 | 2.28 |
| Methanol | 56.3 | 89.0 | 50.1 | 15.10 | 8.50 |
| Ethanol | 41.4 | 89.9 | 37.3 | 16.98 | 7.04 |
| Ether | 6.4 | 52.4 | 3.4 | 21.80 | 1.40 |
| Acetone | 58.2 | 89.9 | 52.3 | 16.36 | 9.52 |
| Acetone-Methanol | 98.6 | 90.2 | 88.9 | 15.54 | 15.32 |

TABLE 2

Lignin Extracted by Hydroxylic Solvents

| ROH | Extracted % |
|----------------------|----------------|
| Water | 16.3 |
| Methanol | 56.3 |
| Ethanol | 41.4 |
| <u>n</u> -Propanol | 81.4 |
| <u>iso</u> -Propanol | 58.1 |
| <u>n</u> -Butanol | 49.4 |
| <u>iso</u> -Butanol | 54.0 |

The general trend of the results of Table 2 is similar to that found by other authors (4,5) although they have been working with different samples of lignin.

As expected, the Klason lignin yields from the water and the ether extracts are low in comparison with that from the original lignin. With solvents like methanol, ethanol, acetone and acetone-methanol there is no significant difference between the Klason lignin yields from the extracts and that from the original lignin. At the same time the MeO-values of such extracts appear to be greater. In the cases of the methanol and ethanol extracts the increased MeO-values of the extracts may be ascribed to two causes: alkylation of reactive hydroxyl groups and partial fractionation of lignins with different MeO-values. In the case of acetone extraction the latter explanation alone is plausible. The fact that the MeO-value of acetone-~~methanol~~ extracts is intermediate between those of extracts with methanol or acetone

alone is not in agreement with the alklylation theory.

The aqueous extract is colloidal and coagulates on prolonged boiling or standing. Such coagulation must occur in the thimble during the extraction. Under standard working conditions the experimental results are readily duplicated but it is clear that the water extracts are not comparable with the other extracts.

Ether extracts are semi-transparent, light coloured, resinous materials melting at 55-57°C. If the extraction is carried out for 40 hours the extract is darker and has a higher melting point (59-63°C.) while the yield rises from 6.4 per cent. to 7.2 per cent. and the Klason lignin yield increases from 52.4 per cent to about 57 per cent. The ether extract is incompletely soluble in ether and some material precipitates in the solvent flask during extraction. The extract is very soluble in lower alcohols, acetone, dioxane, pyridine and acetonitrile, moderately soluble in esters and N-dimethylaniline, partly soluble in water (20%) and slightly soluble in ether and hydrocarbon solvents. Paper partition chromatography, with petrol ether of bp. 80-100°C., or cyclohexane as the mobile phase, established the presence of vanillin and syringaldehyde and two unknown compounds (A and B). The average total concentration of the two former compounds is of the order of 20 per cent with syringaldehyde predominating. The two unknown compounds give spots which may be developed by 2,4-dinitrophenylhydrazine which establishes their carbonylic character. A has $R_F = 0$ (cyclohexane, 20°C.) and may be identical with the aldehyde of Bland and Cohen(6). B with $R_F = 0.25$ may be identical with a postulated unknown aldehyde detected by ultraviolet spectrography in a sample of aldehydes from oxidised lignin (7). On spectrographic

grounds the unknown aldehyde was expected to be the unknown 3-hydroxy-4,5-dimethoxy-benzaldehyde (isosyringaldehyde). A small amount of this substance has been synthesised and was shown to have an R_F value of 0.25. The work involved in this synthesis has little to do with the present problem and will be published on another occasion. This experiment does not prove the identity of B, of course. In fact a spot with $R_F = 0.25$ is obtained with the material which precipitates from alcoholic or aqueous solutions of vanillin on standing exposed to sunlight. If this material is tested immediately after filtering and washing with absolute alcohol to remove traces of vanillin it gives strong aldehydic reactions. On standing the aldehydic properties disappear and the residue is an acid (m.p. 294-296°C. with decomposition) very similar to or identical with dehydrodivanillic acid (m.p. 304°C. with decomposition). The purification of this acid for analytical purposes is difficult and further work is required to clarify its structure. In view of the small quantities and the instability of these unknown aldehydes no further data concerning their identity are available at present but further investigations are in progress and will be reported in due time.

Boiling water in excess removes the aldehydes from the ether extract. The residue is a brown material melting at 70°C. On recrystallizing from benzene pale yellow crystals (m.p. 103-104°C.) are obtained. Boiling with 3 per cent sulphuric acid produces a weak purple fluorescence. The molecular weight by cryoscopy in N-dimethyl aniline is 250 ± 15 . The substance decolorises bromine water. It could be a stilbene derivative and will receive further attention.

Details of experiments with a succession of solvents are given in Table 3. The solvents are listed in order of use. Data on the residue from the acetone-methanol extraction are given for comparison.

TABLE 3

| Treatment | Extracted % | Yield of Klason lignin | | Methoxyl Content of Extract | |
|----------------------|----------------|---------------------------|----------------------------------|--------------------------------|----------------------------------|
| | | based on extract % | based on orig. lignin % | based on extract % | based on orig. lignin % |
| | | | | | |
| Extraction A | | | | | |
| Acetone Methanol | 98.6 | 90.2 | 88.9 | 15.4 | 15.32 |
| Residue | 7.1 | 5.3 | 0.4 | 2.85 | 0.20 |
| Total | 105.7 | - | 89.3 | - | 15.52 |
| Extraction B | | | | | |
| Ether | 4.2 | 51.8 | 2.2 | 21.78 | 0.91 |
| Ethanol | 60.9 | 90.3 | 55.2 | 16.83 | 10.25 |
| Methanol- Acetone | 16.3 | 100.1 | 16.3 | 15.80 | 2.60 |
| Dioxane | 0.4 | - | - | - | - |
| Residue | 17.0 | 78.9 | 13.4 | 12.08 | 1.87 |
| Total | 98.8 | - | 87.1 | - | 15.63 |
| Extraction C | | | | | |
| Ether | 6.4 | 52.4 | 3.4 | 21.80 | 1.40 |
| Ethanol | 60.8 | 90.2 | 54.8 | 16.83 | 10.40 |
| Methanol- Acetone | 16.5 | 98.5 | 16.4 | 15.70 | 2.63 |
| Water | 14.6 | 83.8 | 12.2 | 13.17 | 2.01 |
| Residue | 2.0 | 10.1 | 0.2 | 2.34 | 0.05 |
| Total | 100.3 | - | 87.0 | - | 16.49 |

Although ether extraction removes vanillin, syringaldehyde and other low-molecular weight substances with high methoxyl content the extracts with subsequent organic solvents still have high methoxyl contents. The compositions of the various extracts in the multi-solvent extractions are closely similar to the compositions of extracts with single solvents. The methoxyl content of combined extracts and residue based on original lignin is increased in each experiment which indicates that alkylation takes place to some limited extent and that the partial fractionation of lignins with low and high methoxy values cannot be carried out by this method. The small residue from the extraction with acetone-methanol has characteristics of cellulose while the residues from the multi-solvent experiments represent insoluble lignin. Since lignin is almost quantitatively extracted by acetone-methanol alone, it appears that treatment with other solvents (ether and ethanol) renders the original lignin partially insoluble in the mixed solvent. This cannot be a simple alkylation effect since acetone-methanol is a better solvent than is acetone alone. The solubility of the residue left behind by acetone-methanol in water is due to the colloidal effect mentioned before. No explanation can be given for the reduced acetone-methanol solubility of lignin after extraction with ether and ethanol but similar effects have been observed before (1).

Metal lignates similar to those obtained by Brauns (8) have been prepared by adding metallic salts to a 2 per cent solution of sodium lignate. The lignates of lead, cobalt, mercury, tin, copper, nickel, zinc and aluminium may be formed by this method. Ferrous and manganous lignates could not be prepared in this manner.

The precipitates appeared to be a mixture of metallic hydroxides together with some free lignin since they were appreciably soluble in warm ethanol or acetone whereas the true lignates are insoluble in organic solvents. Lead lignate alone was investigated in some detail owing to its usefulness for the preparation of acetyl lignin. Lead lignate was prepared by adding an excess of a 10-20 per cent. solution of lead acetate to the 2 per cent. sodium lignate solution. A variation of the concentration within these limits did not affect the yield or purity of the lead lignate. The precipitate of lead lignate was filtered, washed with cold water until the filtrates were free from inorganic impurities and dried in an air oven at 50°C. The product is a dense powder, somewhat lighter in colour than the original lignin; it decomposes on heating without melting. The product has a lead content of 39.2 per cent. and a MeO content of 8.73 per cent. against 23.1 per cent. Pb and 17.1 per cent. MeO in Brauns' product. The difference is due to structural causes mainly: Brauns' starting material has 21.5 per cent. MeO against 14.5 per cent. MeO of the Tasmanian alkali lignin. Cobalt lignate prepared by the addition of 10 per cent. cobalt acetate has a cobalt content of 11.7 per cent. A lead content of 39.2 per cent. in lead lignate should correspond to 11.1 per cent. cobalt (found 11.7%) in cobalt lignate and 8.7 per cent. sodium (found 8.9 per cent.) in sodium lignate. This indicates that the metal lignates are formed in accordance with stoichiometric rules.

Lignin was acylated by the following method. Lead lignate (30 g.), acetyl chloride (10.5 g.) and dioxane (200 ml.) were refluxed for 150 minutes. The insoluble lead chloride and

lead lignate were filtered. The filtrate was concentrated and then made up to 150 ml. The filtrate was divided into two portions: A (100 ml.) and B (50 ml.). A was poured into anhydrous ether (400 ml.). The flocculent precipitate of acetyl lignin was filtered, washed with two portions of ether (50 ml. each) and dried in a desiccator over sulphuric acid. The product had a light brown colour and weighed 13.9 g. (Lignin: 72.6 per cent; MeO: 12.1 per cent.; CH_3CO : 16.8 per cent.; m.p. 165-170⁰ C.). Portion B was poured into water (200 ml.) and dried first by washing with a great excess of dry ether and then in a desiccator over sulphuric acid. This method afforded 6.7 g. material which was identical in every respect with the product obtained from portion A. It should be added that the melting point refers to the bulk of the material which contains a small trace of high melting inorganic impurity. Acetyl chloride may be replaced by the equivalent amount of acetic anhydride. This should afford a product of greater purity by method B but no significant difference could be observed.

From the analytical data it is seen that the molecular ratio of methoxy^l to acetyl groups is approximately one. The minimum molecular weight of the acetylated compound is 257 calculated from methoxy^l and 252 from acetyl determinations. One methoxy^l ~~for~~ group for each minimum unit of molecular weight 215 should correspond to a methoxyl content of 14.4 - 14.5 per cent. in the original Tasmanian lignin which is the experimentally found value. The solubility in organic solvents suggests that most of the lignin is present in not too highly polymerised forms. The Pb/MeO ratio in the Tasmanian lead lignate is almost exactly three which suggests a high degree of relative homogeneity compatible with a preponderance of "monomeric" forms only.

In view of earlier experiments in this field (5,9) the possibility of reacting lignin with phthalic anhydride to give useful plastics has been tested. Experimental conditions were varied between the following limits: lignin/phthalic anhydride 2:1 - 1:2; temperature 140 - 200°C.; reaction time 10 - 150 minutes. The first experiments were carried out in the absence of plasticizers. The best results were achieved by heating lignin (55 parts by weight) with phthalic anhydride (45 parts) at 175-185°C. for 60 minutes. The resulting product is thermoplastic (m.p. 178-180°C.), insoluble in ether and hydrocarbon solvents, slightly soluble in esters and chloroform, partially soluble without much decomposition in cold alcohols or acetone and partially soluble with decomposition in hot water or hot ethanol. The product is dark, lustrous and brittle. On plasticizing with 5 per cent n-dibutylphthalate the appearance improves, the material becomes less brittle, softens at 105°C. and melts unsharply over a range up to 140°C. The resistance of the plasticized material to hot water is greatly increased.

Higher ratios of phthalic anhydride to lignin and/or longer periods of heating result in the sublimation of phthalic anhydride from the reaction mixture. Products obtained under these conditions melt around 180°C. and are similar to the phthalyl lignins described by Brauns (5). Castor oil cannot be used as a plasticizer as it gives sticky, semi-solid resins. Substitution of succinic anhydride or maleic anhydride for phthalic anhydride in these experiments gave inferior products. Lack of equipment prevent the investigation of the effect of pressure although this appears to

be the only possibility to obtain lignin resins of potential usefulness by this method.

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NOTES ON ORGANO-COBALT COMPOUNDS

The preparation of some organo-cobalt compounds has been described in earlier publications by D. L. Ingles and J. B. Polya (Nature, 1949, 164, 447; J., 1949, 2280). Dr. Ingles has been engaged on work of a different nature since May, 1949 and the other author lacked the necessary time and research facilities to extend substantially the original investigations. It is expected that suitable equipment will be obtained during 1951 to permit more intensive studies in this field. In the meantime some of the previously described experiments have been repeated. Further analytical data and a few reactions which have been studied with D. A. E. Briggs strengthen the evidence for the organo-metallic character of the compounds under consideration. Further studies on aliphatic organo-cobalt compounds will be reserved for Dr. Ingles who has indicated his readiness to take up this matter again at the next available opportunity. Studies in Hobart will concentrate on the structure and chemistry of organo-cobalt compounds derived from aromatic radicals.

The 1-naphthyl and 2-naphthyl cobalt iodides have been prepared by previously described methods. Although the former observations have been confirmed on the whole in this new series of experiments the published work must be amended in one respect. It was stated that petroleum ether precipitates RCoI_3 (R = 1-naphthyl or 2-naphthyl) from the benzene extract of the reaction products of naphthylmagnesium bromides or iodides with cobalt iodide in ethereal solution whereas precipitation with dioxan yields compounds of the type R_2CoI_2 . In the recent series of experiments all solvents have been highly purified (with the assistance of W. D. Jackson). This reduced the yields of the

petroleum ether precipitation and precipitation with highly purified (crystallized) dioxan afforded first RCoI_3 or $\text{R}_3\text{Co}_2\text{I}_5$ (in the case of $\text{R} = 2\text{-naphthyl}$). The clear filtrate from RCoI_3 deposited small amounts of R_2CoI_2 . It would appear that RCoI_3 is the principal product of the reaction if it is precipitated with petroleum ether. If dioxan is used for precipitation some decomposition occurs. The fact that the decomposition products appears to have simple compositions may indicate that the decomposition occurs in well defined steps and that the later steps of the decomposition proceed more slowly than the earlier ones.

The following analytical data (by D.A.E. Briggs) and yields refer to preparations from naphthylmagnesium bromides and cobalt iodide in ether in M/10 experiments. Previously published data are shown in brackets.

1-Naphthylcobalt iodides

RCoI_3 yield 48% (60%)
decomposes at 153° ($150\text{--}160^\circ$)
yellow-green crystals

| | <u>found</u> | <u>calculated</u> |
|----|--------------|-------------------|
| C | 21.0% | 21.0% |
| H | 1.4% | 1.2% |
| Co | 10.5% | 10.3% |
| I | 66.4% | 67.5% |

R_2CoI_2 yield 2% (55%)
reddish brown crystals

| | <u>found</u> | <u>calculated</u> |
|----|--------------|-------------------|
| Co | 9.7% | 10.3% |
| I | 45.1% | 44.8% |

2-Naphthylmagnesium iodides

RCoI_3 yield 15% (47%)

decomposes at 164-166° (160°)

yellow-green crystals

| | <u>found</u> | <u>calculated</u> |
|----|--------------|-------------------|
| C | 20.8% | 21.0% |
| H | 1.4% | 1.2% |
| Co | 10.0% | 10.3% |
| I | 67.7% | 67.5% |

$\text{R}_3\text{Co}_2\text{I}_5$ yield 15%

greenish grey solid

| | <u>found</u> | <u>calculated</u> |
|----|--------------|-------------------|
| C | 30.2% | 31.6% |
| H | 2.4% | 2.3% |
| Co | 10.8% | 10.3% |
| I | 53.3% | 55.8% |

This material was obviously impure.

R_2CoI_2 yield 1% (less than 1%)

reddish brown crystals

| | <u>found</u> | <u>calculated</u> |
|----|--------------|-------------------|
| Co | 10.7% | 10.3% |
| I | 44.3% | 44.8% |

Reactions of the naphthylcobalt iodides with methyl iodide failed to give the expected methylnaphthalenes and only small amounts of dinaphthyls were recovered from the reaction mixtures.

1-Naphthylcobalt triiodide was successfully reacted with acetyl chloride and benzoyl chloride to the expected ketones. In the 2-naphthyl series the reaction with acetyl chloride was successful

but that with benzoyl chloride failed.

1-Naphthylcobalt triiodide (1.0 g.) was mixed with acetyl chloride (5 cc.). Cobalt chloride and iodine were formed immediately. The mixture was allowed to stand for one hour then heated on a water bath for two hours. More iodine was liberated during heating. After cooling, the mixture was diluted with 15 cc. iced water and extracted with ether. The ether solution was washed with sodium carbonate and sodium thiosulphate solutions and finally with water. After removing the ether the residual brown oil was converted to the semicarbazone of methyl 1-naphthyl ketone, m.p. 235° (after recrystallization from alcohol). The yields were 12-18 mg (3-4.5%).

Similar experiments were carried out with RCoI_3 and $\text{R}_3\text{Co}_2\text{I}_5$ ($\text{R} = 2\text{-naphthyl}$), the latter being a somewhat impure product. In this case the residue left behind after the evaporation of ether was recrystallized twice from petroleum ether ($70\text{-}90^{\circ}$) to give methyl 2-naphthyl ketone, m.p. $52\text{-}53^{\circ}$, identified as the semicarbazone, m.p. $236\text{-}237^{\circ}$. The yields from RCoI_3 were low (6-8%) but one experiment with the impure $\text{R}_3\text{Co}_2\text{I}_5$ gave a yield of 47%.

1-Naphthylcobalt triiodide (1.5 g.) and benzoyl chloride (5 cc.) were allowed to stand for one hour. No visible reaction occurred. On refluxing gently for 90 minutes iodine and cobalt chloride were liberated. The product was worked up as in previous experiments but the residue left behind after the evaporation of the ether was recrystallized 3 times from aqueous alcohol and afforded phenyl 1-naphthyl ketone, m.p. $72\text{-}73^{\circ}$, identified as the oxime, m.p. $140\text{-}141^{\circ}$. D. A. E. Briggs obtained 0.46 g. of the ketone (75%) while the author obtained lower yields of 54-61% in

THE RECOVERY OF ORGANIC MATERIAL FROM COBALT

1-NITROSO-2-NAPHTHOLATE.

By A.D. DARGAVILLE, D.L. INGLES and J.B. POLYA

SUMMARY: The decomposition of cobalt 1-nitroso-2-naphtholate by sodium hydrosulphite gives cobalt sulphide and 1-amino-2-naphthol in good yields. Attempts at the electrolytic oxidation of the latter to 1-nitroso-2-naphthol were unsuccessful.

In the electrolytic process of zinc manufacture traces of cobalt (about 20 mg/litre) must be removed from the zinc sulphate solution before submitting the latter to electrolysis. This is conveniently achieved by precipitating the cobalt with 1-nitroso-2-naphthol prepared in situ from sodium 2-naphtholate and sodium nitrite followed by sufficient sulphuric acid to make the reaction mixture faintly acidic (about 0.1 g. sulphuric acid per litre on completing the reaction). After neutralizing with lime the precipitate is filtered, purified by flotation and worked up for cobalt. The organic material is destroyed in this process. Although 2-naphthol is cheap in comparison with cobalt, the loss of organic material is costly when operations are carried out on a large industrial scale. The present work has been carried out in order to test the possibility of recovering some or all the organic material, preferably in a form in which it could be returned to the process.

Cleavage of cobalt 1-nitroso-2-naphtholate was attempted in semi-quantitative experiments with acids, sodium hydroxide,

hydrogen sulphide, sodium sulphite and sodium bisulphite. Sodium and alcohol led to a darkening of the cobalt complex. Oxalic acid or hydrogen sulphide incompletely precipitated cobalt after this treatment but the organic residue was too complex to permit analysis. In view of the ready reduction of 1-nitroso-2-naphthol to 1-amino-2-naphthol by sodium hydrosulphite (1) the effect of this reagent on the cobalt complex was tried. Cobalt sulphide was recovered in a yield of 91.8% and 1-amino-2-naphthol (as hydrochloride) in a yield of up to 70.0%. The latter compound was oxidized to 1,2-naphthoquinone by Fieser's method (2) with a yield of 93%. Hydroxylamine hydrochloride converted 1,2-naphthoquinone to 2-nitroso-1-naphthol in 70% yield.

2-Nitroso-1-naphthol may be used instead of 1-nitroso-2-naphthol to precipitate cobalt. Regardless of the obviously uneconomic nature of reactions involving the use of hydroxylamine, experiments were carried out on the cleavage of cobalt 2-nitroso-1-naphtholate by sodium hydrosulphite. In this case 93% of the cobalt was recovered as sulphide but only 26% of the theoretical amount of 2-amino-1-naphthol. Oxidation with ferric chloride gave low yields of 1,2-naphthoquinone, 32-36%.

The investigations of Conant and Pratt (3) throw doubt on the possibility of the chemical oxidation of 1,2-naphthol to 1-nitroso-2-naphthol. Electrolytical oxidation appeared to be somewhat more promising. Experiments were carried out with a platinum electrode surrounded by a porous vessel and vigorous stirring of the room between the porous vessel and the anode.

Lead anodes were unsatisfactory in solutions containing chloride or sulphate ions in the presence of which decomposition potentials of 0.50 and 0.88 volts respectively have been noted. In alkaline solutions oxide film formation on the lead anodes made the determination of decomposition potentials difficult. Carbon anodes proved satisfactory with decomposition potentials of 1.43 and 1.66 volts respectively in the presence of chloride and sulphate ions. In alkaline solutions, however, some trouble was experienced owing to the formation of mellitic acid. The hydrochloride and sulphate of 1-amino-2-naphthol were electrolysed in 0.001 molar solutions with current densities of 1.0 - 46.3 microamp/cm², potentials of 1.5-2.0 volts, pH values of 1.0 - 13.0, at room temperature and at 90°C. for 2 1/2 - 15 hours. Between these wide limits substantially the same results were obtained. The solution gradually darkened and a precipitate was formed. This consisted mainly of 1,2-naphthoquinone which occasionally occluded traces of nitrogenous material.

Electrolytic oxidation of both the sulphate and hydrochloride of 1-amino-2-naphthol proceeded smoothly to 1,2-naphthoquinone at potentials below the decomposition potential of the cell.

In acid solutions 1-amino-2-naphthol is stable to atmospheric oxidation and the formation of 1,2-naphthoquinone must be due to electrolytic oxidation. In alkaline solution oxidation to the quinone proceeds in contact with the atmosphere alone. Electrolytic oxidation in alkaline media proceeds much faster than in acidic solutions, in spite of the greater current

density in the latter, and affords red quinoid pigments in addition to 1,2-naphthoquinone.

Addition of cobalt sulphate in 0.0006 - 0.025 molar concentrations did not result in the precipitation of cobalt 1-nitroso-2-naphtholate. In some experiments chloroform was stirred with the solution to facilitate the removal of the cobalt complex. Red solutions obtained in this manner did not contain cobalt. The only effect of addition of cobalt was the gradual deposition of cobalt sesquioxide. The same effect was noted when a saturated solution of 1,2-naphthoquinone was added to solutions of cobaltous salts.

Summarizing, the experiments indicate that the theory of Conant and Pratt on the oxidation of aminonaphthols applies also to electrochemical oxidation. From a practical point of view, it has been demonstrated that the organic portion of cobalt 1-nitroso-2-naphtholate may be recovered in useful yields although not in an immediately useful form for the requirements of the zinc industry. The high price of 1,2-naphthoquinone suggests a profitable outlet for the recovered 1-amino-2-naphthol if the present limited use of the former could be expanded. Alternatively 1-amino-2-naphthol could be converted to phthalic anhydride. On ordinary considerations this may appear as a wasteful process but since the usual process sacrifices the whole of the organic material even a limited recovery might be of economic value. This may be particularly so if the implied saving of naphthalene derivatives would help to reduce dollar expenditure.

Experimental

Cobalt 1-nitroso-2-naphtholate (24 g.) is dried and suspended in 5N sodium hydroxide solution (300 cc.). Sodium hydrosulphite (60 g.) is stirred in at 35°. An exothermic reaction raises the temperature to 65°-70°. After stirring for 30 minutes the cobalt sulphide is filtered off and washed with warm water. Acidification of the filtrate with hydrochloric acid followed by recrystallization from hot water afford 14.2 - 17.1 g. 1-amino-2-naphthol hydrochloride (58-70%).

Cobalt 2-nitroso-1-naphtholate (g.) by the same procedure gives 2-amino-1-naphthol hydrochloride, 2.3 g. (28.3%).

The authors wish to acknowledge a generous grant from the Electrolytic Zinc Co. of A/asia Ltd. and a Commonwealth Research Grant.

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Colorimetric micro-determination of cobalt.

by J.B. Polya and B. Wilson

Benzidine and dimethylglyoxime in alcoholic solution produce a reddish brown colour with neutral or slightly acid solutions of cobalt. (Brasley - Hobart, J. Am. Chem. Soc. 43 482, 1921; Chiarottino, Ind. Chimica 8, 32, 1933). In dilute solutions the Lambert-Beer law is obeyed accurately enough. Nickel and copper are precipitated by the reagent and may be removed by filtration. Iron interferes but more than 50 parts of iron are needed to give a colour equivalent to that produced by one part of cobalt. The method is useful when reasonably pure cobalt compounds are being investigated (diffusion experiments, organic cobalt compounds etc.).

Procedure:

Benzidine (0.5 g.) and dimethylglyoxime (0.25 g.) are dissolved in 95% ethanol to make 100 cc. 5 cc. of the reagent is mixed with 5 cc. of neutral cobalt solution and diluted to 25 cc. with absolute alcohol. A mixture of 35 cc. reagent, 5 cc. distilled water and 15 cc. absolute alcohol is used as a blank. The intensity of the colour is measured with a Hilger absorptiometer. 4 cm cells are used. Blue filters (H 455) give the best results: with a green filter the readings are too low and with a purple filter the colour versus concentration curve is not steep enough. It is best to carry out the assay one hour after producing the colour as the intensity versus time change is negligible then. For approximative analysis the following table can be used:

| <u>Drum reading</u> | <u>mg Co/ cc.</u> |
|---------------------|-------------------|
| 0.05 | 0.001 |
| 0.10 | 0.002 |
| 0.20 | 0.004 |
| 0.30 | 0.006 |
| 0.40 | 0.008 |
| 0.50 | 0.010 |
| 0.60 | 0.012 |
| 0.70 | 0.015 |
| 0.80 | 0.019 |
| 0.90 | 0.022 |
| 1.00 | 0.026 |
| 1.10 | 0.030 |
| 1.20 | 0.035 |
| 1.30 | 0.040 |
| 1.40 | 0.044 |
| 1.50 | 0.051 |

For accurate purposes the instrument is calibrated with a standard cobalt solution diluted to 0.005% or lower concentrations (higher concentrations give unreliable results). Amounts between 20 gammas and a milligram of cobalt can be estimated with the usual microchemical accuracy.

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THE PREPARATION OF 2-NITRO-4-AMINOPHENYLARSONIC
ACID

By J. B. Polya, Chemistry Department, University
of Tasmania, Hobart.

Summary: Full experimental details are reported for the preparation of 2-nitro-4-amino-phenylarsonic acid from p-phenylenediamine. Although the overall yield is 17-22% only the method is more satisfactory than other syntheses.

In the course of investigations of the chemistry of substituted arsanilic acids with Dr. W. Freund substantial amounts of 2-nitro-4-aminophenylarsonic were required. This substance may be synthesized by converting p-phenylenediamine (I) to diacetyl p-phenylenediamine (II), nitrating the latter to its mononitro derivative (III) which on controlled hydrolysis gives 2-nitro-4-acetamidobeniline (IV). The latter may be converted to the corresponding arsonic acid (V) and then hydrolysed to 2-nitro-4-aminophenylarsonic acid (VI) as described by Haythornthwaite (1) or the two operations may be carried out in succession without isolating V (2). In either case the preparation of IV is the most difficult step. Although the preparation of this compound has been described before, the published experimental details are scanty and yields are not given in the literature. The combination of the Bart reaction and hydrolysis in one step has been recorded in a patent claim only

which does not give sufficient directions to isolate the required compound.

The conversion of I into II has been reported very briefly by Biedermann and Ledoux (3) without any quantitative data save for an approximate melting point determination. The following method gives nearly quantitative yields. Glacial acetic acid (1500 g.) and I (432 g.) are mixed in a 3 litre round-bottom flask attached to a 24" Young-Thomas column; 250 cc. of the solvent is distilled (at about 108°) over a period of 2 hours, fresh glacial acetic acid (300 cc.) is added and the distillation is continued until the distillate amounts to 400-410 cc. At this stage the mixture begins to bump owing to the separation of solid matter. After cooling to room temperature the grey-violet crystals are filtered and washed with ether. Air drying on porous plates affords 630-644 g. material melting at $301-303^{\circ}$ which is satisfactory for the subsequent operations. Concentration of the mother liquor to 500 cc. affords more material of similar quality to give a total yield of 680-690 g. (89 - 90%). Further concentration of the mother liquor affords small amounts of impure II. The crude product may be purified by recrystallization from 60 parts by weight of glacial acetic acid and treatment with Norite. The pure material melts at $303-304^{\circ}$ but the first crop is obtained in a yield of 25-26%. The crude product may

be obtained in slightly higher yields (91-93%) by increasing the amount of glacial acetic acid to 2300-2400 cc. and the colour may be improved by carrying out the acetylation in the presence of 0.1 g. hydroquinone.

The nitration of II to III has been described by Biedermann (4), Ladenburg (5) and Bülow and Mann (6). Apart from the statement of Ladenburg that the yields are good, these authors did not state their yields. The recommended procedure is based on the method of Bülow and Mann. Ice-cold sulphuric acid, sp. gr. 1.84 (3840 g.) is well stirred in a 3 litre round-bottom flask cooled in an ice-salt bath and II (384 g.) is added in small portions over a period of about one hour so as to keep the temperature between 15° and 20° . The resulting viscous solution is cooled to -10° in a carbon dioxide - ether bath and is nitrated with a chilled mixture of nitric acid, sp. gr. 1.40 (192 g.) and sulphuric acid, sp. gr. 1.84 (384 g.). The nitrating mixture is added slowly with good stirring over a period of 50-60 minutes to maintain the temperature of the reaction mixture between -7° and -4° . After adding all the nitrating mixture the flask is allowed to stand at -10° for 2 hours. It is poured then on 20 lbs. of finely crushed ice, diluted with 20 litres of cold water and filtered by suction as rapidly as possible. The yellow precipitate is washed on the filter funnel

with 10 litres each of cold water, $\frac{1}{2}\%$ sodium bicarbonate solution and cold water again. The precipitate is transferred to a beaker and stirred with 3 litres of cold $\frac{1}{2}\%$ sodium bicarbonate solution for 5 minutes, filtered and washed rapidly with 5 litres of cold water in 3 portions. Some material is lost in these operations which, however, were found to be essential to remove traces of inorganic acids from the material. It is advisable to continue the washing of the precipitate until the test for sulphate in the filtrate becomes negative. If this is omitted the preparation may deteriorate on keeping and may undergo partial hydrolysis during recrystallization from water. There is some decomposition if the material is dried at 105° . It is best to air-dry on porous plates and to complete the drying in a vacuum desiccator over caustic alkali. The crude product is obtained in a yield of 397-417 g. (84-88%), melts at $180-182^{\circ}$ and may contain up to 1.2% moisture. This material is satisfactory for further operations but may be recrystallized from 40 parts of boiling water with a loss of 8-11%. Thermic analysis shows that III has two crystalline modifications, one with the previously recorded melting point of 186° (5) and an unstable modification melting at 174° .

The hydrolysis of III by concentrated aqueous ammonia (6) gave negligible yields of the order of 1% (cf. 4). Boiling the substance with 8 parts of

10% aqueous potassium hydroxide for 10-20 minutes afforded nitro-p-phenylenediamine, m.p. 134° , in yields fluctuating around 40%. Hydrolysis by dilute mineral acids could not be controlled and hydrolysis by borax, trisodium phosphate or sodium sulphite was incomplete. Hydrolysis by dilute barium hydroxide (6) gave consistent yields 58-62%. The following procedure was found to be satisfactory. III (100 g.) was stirred into 5% aqueous sodium hydroxide (4000 cc.) at 85° , kept at $85-90^{\circ}$ for 50 minutes and allowed to cool in the refrigerator over night. The precipitate was filtered and washed on the filter with N/10 hydrochloric acid (50 cc.) to remove traces of nitro-p-phenylenediamine. In addition to 62-67 g. material obtained in this way and additional 2-3 g. could be recovered by saturating the mother liquor with sodium chloride. The crude yield of IV is 64-70 g. (78-85%), m.p. $181-2^{\circ}$. Recrystallization from 30 parts of boiling water reduces the yield to 60-64% and raises the melting point to 189° . Since the melting points of III and IV are closely similar and even the colours may be similar depending on the size of crystals (III : pale yellow to orange red; IV : bronze red to brownish purple) analytical checking of the product is essential. N found 21.41%, calculated 21.54%; molecular weight found 203 ± 11 , calculated 195.

The conversion of IV to V by Haythornthwaite's

method (1) has been repeated and yields fluctuating around 46% have been confirmed. However, the losses on hydrolysing V to VI by dilute alkali and precipitating at pH 5.0-5.2 were considerable and the overall yields of VI from IV varied between 22% and 27%. Hydrolysis with dilute sulphuric acid (2) gave better yields (32-35%) but the product was contaminated with m-nitroaniline (1). A modification of Ger. Pat. 267307 appears to be satisfactory. IV (65 g.) is slowly stirred into concentrated hydrochloric acid (280 cc.) with external ice-salt cooling. On adding ice-cold water (400 cc.) a stiff paste of brilliant red colour is formed. This is diazotized and stirred into a solution of sodium arsenite (134 g.) in water (300 cc.) at 20°. The temperature rises to about 25° and most of the reaction is complete in one hour. The mixture is heated to 60° for one hour and filtered. Refluxing for 4-5 hours followed by chilling should afford VI according to the patent. This, however, does not occur to any great extent. It is best to add concentrated hydrochloric acid (100 cc.), concentrate to 600 cc. on a water bath, adjust the pH to 5.0-5.2 by the careful addition of sodium carbonate and sodium acetate and the volume to about 700 cc. After cooling the precipitate is filtered and the filtrate is concentrated to 500 cc., adjusting the pH to 5.0-5.2 if necessary. On chilling a mixture of organic and inorganic material is

obtained. The former is extracted with methanol in a Soxhlet apparatus. The first crop and the contents of the methanol extract are combined and recrystallized from 50 parts of boiling water with Norite. The filtrate is concentrated to one quarter of its original volume. On chilling 33-42 g. (36-46%) VI is obtained. The orange-red substance darkens slowly from 235-240° and melts unsharply with decomposition at 255-258°. N found 10.62%, calculated 10.69%; As found 27.97%, calculated 28.60%. Attempts were made to prepare V by Scheller's method following Doak's procedure (7) which has been found satisfactory for the preparation of some substituted phenylarsonic acids in this laboratory but the yield was inferior to that of Haythornthwaite (1) and the product was contaminated by arsenic trioxide.

References:

- 1) Haythornthwaite : J., 1929, 1011.
- 2) Höchst. Farbwerke, German Patent 267307 (1911).
- 3) Biedermann, Ledoux : Ber., 1874, 7, 1531.
- 4) Biedermann : Ber., 1874, 7, 1533.
- 5) Ladenburg : Ber. 1884, 17, 147.
- 6) Bulow, Mann : Ber., 1897, 30, 977.
- 7) Doak : J. Amer. Chem. Soc., 1940, 62, 167.